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Early life exposure to air pollution and cell-mediated immune responses in preschoolers

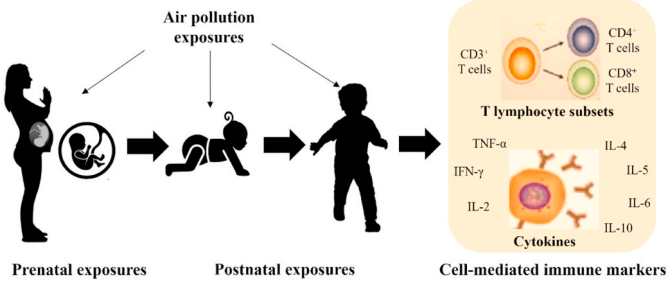
Yan-Ling Deng [a,b,1], Jia-Qiang Liao [b,1], Bin Zhou [c], Wen-Xin Zhang [b], Chong Liu [a,b], Xiao-Qiong Yuan [d], Pan-Pan Chen [a,b], Yu Miao [a,b], Qiong Luo [a,b], Fei-Peng Cui [a,b], Min Zhang [a,b], Sheng-Zhi Sun [e], Tong-Zhang Zheng [f], Wei Xia [b], Yuan-Yuan Li [b], Shun-Qing Xu [b,**], Qiang Zeng [a,b,f,*]

[a] *Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China*
[b] *Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China*
[c] *Wuhan Medical and Health Center for Women and Children, Wuhan, Hubei, China*
[d] *Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095, Jiefang Avenue, Wuhan, Hubei, PR China*
[e] *Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA*
[f] *Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA*

HIGHLIGHTS

- Effects of air pollution exposure on cell-mediated immune responses were estimated.
- Late pregnancy air pollution exposures were positively related to %$CD3^+$ cells.
- Early pregnancy air pollution exposures were related to reduced %$CD3^+$ and %$CD3^+CD8^+$ cells.
- Postnatal air pollution exposures were positively associated with certain cytokines.
- There were sex-specific associations between air pollution exposures and immune responses.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Exposure to air pollution has been linked with altered immune function in adults, but little is known about its effects on early life. This study aimed to investigate the effects of exposure to air pollution during prenatal and postnatal windows on cell-mediated immune function in preschoolers.
Methods: Pre-school aged children (2.9 ± 0.5 y old, n = 391) were recruited from a mother-child cohort study in Wuhan, China. We used a spatial-temporal land use regression (LUR) model to estimate exposures of particulate

* Corresponding author. Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China.
** Corresponding author. Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
E-mail addresses: xust@hust.edu.cn (S.-Q. Xu), zengqiang506@163.com, qiang_zeng@brown.edu (Q. Zeng).
[1] Joint as the first co-authors.

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T lymphocyte subsets
Cytokines
Prenatal
Postnatal

matter with aerodynamic diameters ≤2.5 μm ($PM_{2.5}$) and ≤10 μm (PM_{10}), and nitrogen dioxide (NO_2) during the specific trimesters of pregnancy and the first two postnatal years. We measured peripheral blood T lymphocyte subsets and plasma cytokines as indicators of cellular immune function. We used multiple informant models to examine the associations of prenatal and postnatal exposures to air pollution with cell-mediated immune function.

Results: Prenatal exposures to $PM_{2.5}$, PM_{10}, and NO_2 during early pregnancy were negatively associated with % $CD3^+$ and %$CD3^+CD8^+$ cells, and during late pregnancy were positively associated with %$CD3^+$ cells. Postnatal exposures to these air pollutants during 1-y or 2-y childhood were positively associated with IL-4, IL-5, IL-6, and TNF-α. We also observed that the associations of prenatal or postnatal air pollution exposures with cellular immune responses varied by child's sex.

Conclusions: Our results suggest that exposure to air pollution during different critical windows of early life may differentially alter cellular immune responses, and these effects appear to be sex-specific.

1. Introduction

Air pollutants are a ubiquitous and complex mixture of gases such as nitrogen dioxide (NO_2) and particulates such as inhalable particles with aerodynamic diameters ≤10 μm (PM_{10}) and ≤2.5 μm ($PM_{2.5}$) (Chiu et al., 2014; Morales et al., 2014; Liu et al., 2019). Exposure to air pollution has been shown as one of the main environmental risk factors for the global burden of disease (Forouzanfar et al., 2016). Mounting evidence has suggested that air pollution exposures are related to immune-mediated diseases including asthma and allergy (Bowatte et al., 2015; Khreis et al., 2017), type 1 diabetes mellitus (Elten et al., 2020), multiple sclerosis (Palacios et al., 2017), systemic lupus erythematosus (Alves et al., 2018), and rheumatoid arthritis (De Roos et al., 2014). Although the underlying mechanisms for these immune-mediated diseases are still elucidated, an alteration in cell-mediated immune function has been proposed to be one of the key pathways (Yang et al., 2014).

Experimental studies have found that air pollutants can trigger cell-mediated immune responses (Sasaki et al., 2009; Saunders et al., 2010). In murine models, maternal exposure to PM inhibited T lymphocyte subsets, as reflected by decreased splenic $CD3^+CD8^+$ and $CD3^+CD4^+$ cells in male offspring and reduced thymic $CD4^+CD25^+$ cells in female offspring (Chen et al., 2018a). A mouse model in pregnant C57BL/6 mice showed that maternal exposure to diesel exhaust particles enhanced the expression of type 1 helper T cell (Th1) cytokines [e.g., tumour necrosis factor alpha (TNF-α)] and Th2 cytokines [e.g., interleukin-6 (IL-6), IL-5, and IL-4] in offspring (Manners et al., 2014). Moreover, *in-vivo* and *in-vitro* studies have suggested that exposure to PM and PM_{10} can inhibit Th1 cytokines but induce Th2 cytokines, as evidenced by decreased interferon-γ (IFN-γ) (Hong et al., 2013; Wang et al., 2013) and increased IL-10, IL-6, IL-5, and IL-4 (Fujii et al., 2002; Porter et al., 2007; Hong et al., 2013; Matthews et al., 2016).

A number of epidemiological studies have suggested that air pollution exposures may alter cellular immune function. Some studies observed positive associations of air pollution (e.g., $PM_{2.5}$, NO_2, and sulfur dioxide) with T lymphocyte subsets (Pope et al., 2016; Gao et al., 2019) and cytokines in adults (Panasevich et al., 2009), while others showed negative (Mostafavi et al., 2015) or null associations (Williams et al., 2009; Tsai et al., 2019). However, the evidence whether exposure to air pollution during early life affects cell-mediated immune responses is still scarce (Leonardi et al., 2000; Hertz-Picciotto et al., 2005; Li et al., 2019). Early life such as the prenatal period and first few postnatal years is more susceptible to environmental pollutants since the immune system is innate and still in development (Hertz-Picciotto et al., 2008). The alternation of immune function during the critical early life may have far-reaching consequences for health in adults (Dietert et al., 2000).

The aim of this study was to investigate the associations of exposure to air pollution across critical time windows of early life with cell-mediated immune function in pre-school children. We assessed $PM_{2.5}$, PM_{10}, and NO_2 exposures during the specific trimesters of pregnancy and the first two postnatal years in a mother-child cohort study, China. T lymphocyte subsets and cytokines were measured as markers of cell-mediated immune function.

2. Materials and methods

2.1. Participants

During September 2012 and October 2014, the mother-child pairs were selected from an ongoing prospective birth cohort study at the Wuhan Children's Hospital, China. Detailed descriptions for the inclusion criteria have been provided previously (Liao et al., 2018; Zeng et al., 2020). In brief, pregnant women who were residents of Wuhan and gave birth at the study hospital with a singleton pregnancy and without birth defects were included in this cohort study. From the eligible pregnant women, a follow-up study of environmental chemical exposures and children's immune function was carried out between August 2017 and August 2018 (Zeng et al., 2020). A total of 407 children aged around 3 years old were given a physical examination before admission to pre-school. These children were asked to provide peripheral blood samples for immune function evaluation in the follow-up study. After excluding the missing data of residence for the participants (n = 16), the final analysis consisted of 391 mother-child pairs in this study. All the participants gave a written informed consent at enrolment. The ethics committees of Tongji Medical College (number [2012]14) and Wuhan Children's Hospital approved the study protocol (number 2010009).

2.2. Assessment of air pollution exposures

The concentrations of ambient air pollutants for NO_2, PM_{10}, and $PM_{2.5}$ were collected from the Wuhan Environmental Monitoring Center. The ArcGIS version 9.3 was used to geocode residential home addresses. The residential home addresses were obtained by a questionnaire and updated at each subsequent study visit. The area of each land use type was obtained from the Landsat-7 Enhanced Thematic Mapper-Plus ETM + data. The road-related predictor variables were derived from digital road network data of OpenStreetMap. The types of enterprise-related air pollution were obtained from the Wuhan Environmental Protection Agency. The daily average temperatures were obtained from national routine monitoring weather stations and calculated by inverse-distance weighing method.

We assessed the prenatal and postnatal average daily exposures of NO_2, $PM_{2.5}$, and PM_{10} at residential address using short-terms (weeks) and long-terms (seasons) variations adjusted spatial-temporal land use regression (LUR) model (with 12 × 4 knots of natural cubic splines per year), as previously described (Kloog et al., 2012; Liao et al., 2018). The LUR model was quantified by the "out of sample" 10-folds cross-validation (CV). We used 70% of data as training the LUR model and 30% of data as validating the results of model fitting in each fold CV assessment. We compared the differences between the measured values and prediction values of models. The results showed that our model had a good variability to predict the prenatal and postnatal concentrations of NO_2, $PM_{2.5}$, and PM_{10}: the R^2 for the model explained variance was 54.8%–71.6% and the R^2 for the average 10-fold CV was 52.0%–73.0%.

The prenatal and postnatal average concentrations of NO_2, $PM_{2.5}$,

and PM_{10} were calculated by the trimesters and first two postnatal years, respectively. The first trimester was defined from conception to 13 weeks of pregnancy, the second trimester from 14 to 27 weeks of pregnancy, and the third trimester from 28 weeks of pregnancy to delivery. The 1-y childhood was defined from birth to 1 year of children and the 2-y childhood from 1 year to 2 years of children.

2.3. Measurements of cell-mediated immune markers

We collected peripheral blood samples from children at the health clinics by tubes with potassium ethylenediaminetetraacetic acid (EDTA). The collected tubes with ice packs at each day were used to measure T lymphocyte subsets within 24 h at Wuhan Maternal and Child Healthcare Hospital laboratory. The remaining blood samples were centrifuged to obtain the plasma and then stored at −80 °C for cytokine analyses.

T lymphocyte subsets and cytokines as markers of cell-mediated immune function were measured as has been detailed previously (Zeng et al., 2020). All tests and quality controls were performed in accordance with the manufacturer's instructions. T lymphocyte subsets were measured using BD FACSCanto™ II flow cytometer with BD Multitest™ IMK kit and BD Trucount tubes (Becton, Dickinson and Company, San Jose, USA). We reported here the absolute counts and percentages of $CD3^+$, $CD3^+CD8^+$, and $CD3^+CD4^+$ cells. We calculated the $CD4^+/CD8^+$ as the ratio of $\%CD3^+CD4^+$ cells to $\%CD3^+CD8^+$ cells. Cytokines including IL-10, IL-6, IL-5, IL-4, IFN-γ, TNF-α, and IL-2 in plasma were assessed using Luminex® FLEXMAP 3D® instrument with MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel 96-Well Plate Assay (EMD Millipore Corporation, Billerica, USA). The coefficients of variation and recoveries for cytokines ranged from 0.03% to 10.36% and from 98.89% to 102.33%, respectively. The limits of detections (LODs) for these plasma cytokines ranged from 0.18 to 1.50 pg/mL. A total of 329 children had the available data of plasma cytokines in the current study.

2.4. Covariates

Information on health-related behaviors and socio-demographic characteristics was collected by trained nurses using a questionnaire at enrolment. The collected information included maternal age, education levels, pre-pregnancy body mass index (BMI), household income, physical activities, and passive smoking during pregnancy. Exposure to secondhand smoke at home and/or at work during pregnancy was defined as passive smoking (Vardavas and Panagiotakos, 2009). No mothers reported that they had the habits of active smoking and alcohol drinking during pregnancy. Information on gestational age, delivery mode, parity, birth length, birth weight, child's sex, and date of birth was collected from medical records.

2.5. Statistical analysis

Descriptive statistics were performed on the basic characteristics, concentrations of NO_2, PM_{10}, and $PM_{2.5}$, and cell-mediated immune markers of the study population. The concentrations of plasma cytokines (IL-10, IL-6, IL-5, IL-4, IL-2, TNF-α, and IFN-γ), $CD4^+/CD8^+$ ratio, the absolute counts of $CD3^+$, $CD3^+CD8^+$, and $CD3^+CD4^+$ cells showed skewed distribution and thus were ln-transformed to assure normality for the following analyses. Spearman correlation coefficients and Kruskal-Wallis H test were calculated among air pollutants by different time windows. All the statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.6.0. The *P*-values of hypothesis tests <0.05 were considered as statistically significant.

We used multiple informant models with generalized estimating equations to estimate the effects of prenatal (the first, second, and third trimester of pregnancy) and postnatal (1-y and 2-y childhood) air pollution exposures on T lymphocyte subsets and plasma cytokines (Sanchez et al., 2011). This model treated prenatal and postnatal air exposure levels at different windows as informants and simultaneously assessed the associations of prenatal and postnatal each 10 $\mu g/m^3$ increase in NO_2, $PM_{2.5}$, and PM_{10} with immune markers at each exposure window. Additionally, this model supplied an approach to test differences in associations of prenatal and postnatal air pollution exposures with cellular immune responses at different time windows. The null hypothesis for the test was that the coefficients for air pollution exposures were equal at each exposure window. The regression coefficients for those natural ln-transformed immune markers were calculated as $[\exp(\beta) - 1] \times 100\%$ to obtain percent changes for ease of interpretation.

Directed Acyclic Graphs (DAG) methods based on a six-step process were used to select the potential confounders (Shrier and Platt, 2008). These covariates were finally included in multiple adjusted models as the following: child's age (days) and maternal age (years) as continuous variables; child's sex (boys vs. girls), maternal education levels (less than college vs. college and above), passive smoking during pregnancy (no vs. yes), parity (primiparous vs. multiparous), delivery mode (vaginal vs. cesarean), and season of delivery (September–February vs. March–August) as dichotomous variables; household income ($<$50000, 50000-100000, or $\geq$100000 yuan/year) and maternal physical activities during pregnancy (never, 1–4 days each week, or $\geq$5 days each week) as ordinal variables.

Because cadmium exposure has been reported to be associated with altered cellular immune responses in our previous study (Zeng et al., 2020), a sensitivity analysis was performed by additionally adding the adjustment for maternal urinary cadmium or children's plasma cadmium. In addition, because a previous study indicated that infant sex may modify the association between maternal exposure to air pollution and immune cell development (Chen et al., 2018a), we further investigated child's sex as a potential effect modifier. A multiplicative interaction term between child's sex and air pollutants was included in the models. The Wald test was used to estimate *P*-values for interaction (Kaufman and MacLehose, 2013). The adjusted covariates except for child's sex in the gender-stratified analyses were the same as above mentioned.

3. Results

3.1. Population characteristics

The basic characteristics of mother-child pairs are displayed in Table 1. Among the children, 210 (53.7%) were boys, and 141 (36.1%) were delivered in summer. The mean (SD) age, gestational age, birth weight, and birth length were 2.9 (0.5) years, 39.3 (1.1) weeks, 3378.1 (395.5) g, and 50.3 (1.5) cm, respectively. For the mothers, the mean (SD) pre-pregnancy BMI and maternal age were 21.2 (3.0) kg/m^2 and 28.9 (3.5) years, respectively. Most of the mothers were primiparous (82.4%) and cesarean (80.6%), and performed physical activities over 5 days each week (74.9%) during pregnancy. Less than half of the mothers were well educated (46.5%) and 28.6% of them were passively exposed to smoking during pregnancy.

3.2. Distribution of cell-mediated immune markers

Table 2 displays the distribution of cell-mediated immune markers. Among the T lymphocyte subsets, the median absolute counts of $CD3^+$, $CD3^+CD8^+$, and $CD3^+CD4^+$ cells were 2518.60, 844.40, and 1413.52 cells/μL, respectively; the median $\%CD3^+$ cells, $\%CD3^+CD8^+$ cells, $\%CD3^+CD4^+$ cells, and $CD4^+/CD8^+$ ratio were 68.49, 23.14, 38.10, and 1.61, respectively. For the plasma cytokines, the median concentrations of IL-4 were the highest (27.46 pg/mL), followed by IFN-γ (16.25 pg/mL), IL-10 (8.66 pg/mL), TNF-α (7.46 pg/mL), IL-2 (4.74 pg/mL), IL-5 (3.88 pg/mL), and IL-6 (3.17 pg/mL).

Table 1
Characteristics of mother-child pairs included in this analysis (n = 391).

Characteristic	Mean ± SD or n (%)
Children	
Age (years)	2.9 ± 0.5
Birth weight (g)	3378.1 ± 395.5
Birth length (cm)	50.3 ± 1.5
Gestational age (week)	39.3 ± 1.1
Sex	
Boys	210 (53.7)
Girls	181 (46.3)
Season at delivery	
Spring	124 (31.7)
Summer	141 (36.1)
Autumn	78 (19.9)
Winter	48 (12.3)
Mothers	
Age (years)	28.9 ± 3.5
Pre-pregnancy BMI (kg/m^2)	21.2 ± 3.0
Parity	
Primiparous	322 (82.4)
Multiparous	69 (17.6)
Delivery mode	
Vaginal	76 (19.4)
Cesarean	315 (80.6)
Education levels	
Less than college	209 (53.5)
College or above	182 (46.5)
Household income (yuan/year)	
<50000	111 (28.6)
50000–100000	173 (44.6)
≥100000	104 (26.8)
Passive smoking during pregnancy	
No	279 (71.4)
Yes	112 (28.6)
Physical activities during pregnancy	
Never	43 (11.0)
1–4 days per week	55 (14.1)
≥5 days per week	292 (74.9)

Abbreviations: SD: standard deviation; BMI: body mass index.
[a]3 missing household income and 1 missing physical activities during pregnancy.

3.3. Distribution of air pollutants levels and their correlations

The distribution of prenatal and postnatal air pollutant levels at different time exposure windows is displayed in Fig. 1. Overall, there were decreasing trends of average levels for NO_2, PM_{10}, and $PM_{2.5}$ from prenatal periods (the first, second, and third trimester) to postnatal periods (1-y childhood and 2-y childhood) (all $P < 0.001$, data not shown). Moreover, moderate or strong correlations among air pollutants were observed within each time window ($r_s = 0.45$ to 0.91) (Table S1), and inconsistent correlations were observed across different time windows ($r_s = -0.57$ to 0.63) (Table S2).

3.4. Associations between air pollution exposures and cell-mediated immune markers

Adjusted percent changes (95% CI) in children's percentage of T lymphocyte subsets in relation to air pollution exposures at different time windows are shown in Fig. 2. Each 10 μg/m^3 increment in prenatal exposure to $PM_{2.5}$, PM_{10}, and NO_2 during early pregnancy was associated with 2.69% (95% CI: −4.14%, −1.24%), 1.84% (95% CI: −3.38%, −0.31%), and 5.95% (95% CI: −9.89%, −2.01%) decreases in %CD3$^+$ cells, respectively. Similar negative associations were observed for %CD3$^+$CD8$^+$ cells with percent changes of −1.72% (95% CI: −3.11%, −0.34%), −1.37% (95% CI: −2.81%, 0.08%), and −3.72% (95% CI: −7.43%, −0.01%), respectively. In contrast, each 10 μg/m^3 increment in prenatal exposure to $PM_{2.5}$, PM_{10}, and NO_2 during late pregnancy was associated with 1.82% (95% CI: 0.08%, 3.56%), 3.10% (95% CI: 1.07%, 5.14%), and 5.53% (95% CI: 0.57%, 10.50%) increases in %CD3$^+$ cells, respectively. Moreover, a 10 μg/m^3 increment in PM_{10} exposure during the third trimester was associated with 2.04% (95% CI: 0.12%, 3.96%) increase in %CD3$^+$CD8$^+$ cells. However, we did not found clear associations between air pollution exposures and the absolute counts of T lymphocyte subsets (see Table S3).

Adjusted percent changes (95% CI) in children's cytokines in relation

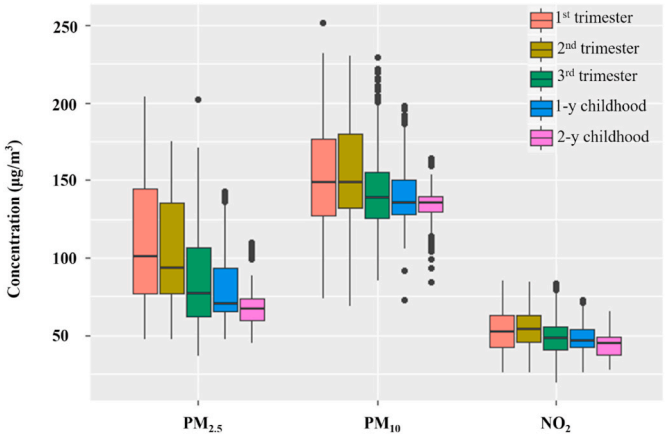

Fig. 1. Distribution of air pollutant levels (μg/m^3) at different time exposure windows. Box plots: minimum, 25% percentile, median, 75% percentile, and maximum.

Table 2
Distribution of peripheral blood T lymphocyte subsets and plasma cytokine concentrations among the children.

Variables	Mean ± SD	Min	25th	50th	75th	Max
Peripheral blood T lymphocyte subsets (n = 391)						
CD3$^+$ (cells/μL)	2635.31 ± 850.19	616.64	2115.87	2518.60	3026.51	7213.22
%CD3$^+$	68.37 ± 5.59	47.73	64.67	68.49	72.38	82.63
CD3$^+$CD4$^+$ (cells/μL)	1472.94 ± 526.04	308.26	1137.91	1413.52	1703.49	4046.69
%CD3$^+$CD4$^+$	38.09 ± 6.08	15.53	34.31	38.10	42.15	58.75
CD3$^+$CD8$^+$ (cells/μL)	909.92 ± 360.61	214.79	685.39	844.40	1091.80	2650.23
%CD3$^+$CD8$^+$	23.44 ± 5.27	9.94	19.78	23.14	26.66	50.70
CD4$^+$/CD8$^+$	1.74 ± 0.60	0.49	1.34	1.61	2.10	5.43
Cytokines (n = 324)						
IL-2 (pg/mL)	4.98 ± 1.74	1.70	3.76	4.74	5.89	12.58
IL-4 (pg/mL)	31.18 ± 21.23	9.91	23.09	27.46	34.10	338.71
IL-5 (pg/mL)	3.95 ± 1.17	1.74	3.15	3.88	4.64	13.43
IL-6 (pg/mL)	9.68 ± 50.02	1.18	2.52	3.17	5.26	785.92
IL-10 (pg/mL)	9.58 ± 6.60	0.87	6.71	8.66	11.23	84.13
IFN-γ (pg/mL)	17.34 ± 6.41	6.47	13.52	16.25	19.54	74.81
TNF-α (pg/mL)	13.49 ± 15.15	3.14	5.54	7.46	15.24	102.50

Abbreviations: SD: standard deviation; Min: minimum; Max: maximum; IL-2: interleukin-2; IFN-γ: interferon-γ; TNF-α: tumour necrosis factor alpha.

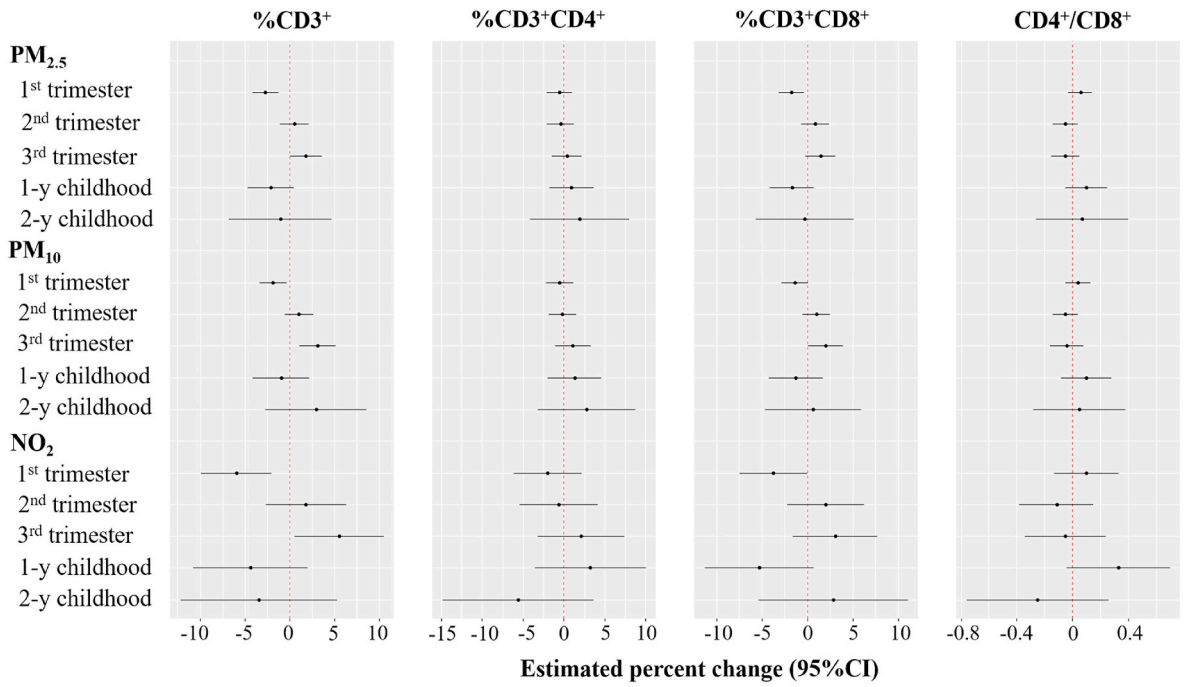

Fig. 2. Adjusted percent changes (95% CIs) in children's percentage of peripheral blood T lymphocyte subsets associated with per 10 μg/m^3 incremental change in air pollution exposure at different time windows. All models were adjusted for children age, children sex, season at delivery, maternal age, parity, delivery mode, educational levels, household income, passive smoking during pregnancy, and physical activities during pregnancy.

to exposure to air pollution at different time windows are presented in Fig. 3. Except that a 10 μg/m^3 increment in prenatal $PM_{2.5}$ exposure during the first trimester was associated with an increase in IL-4 of 0.11% (95% CI: 0.01%, 0.21%), we did not found any significant associations of prenatal other air pollutant exposures with plasma cytokines. However, we observed that postnatal air pollution exposures were related to increased plasma cytokines. Each 10 μg/m^3 increment in $PM_{2.5}$ exposure during 1-y childhood was associated with increases in IL-6 of 0.59% (95% CI: 0.17%, 1.03%) and TNF-α of 0.49% (95% CI: 0.11%, 0.87%), and the increasing trends persisted during the 2-y childhood. Similarly, each 10 μg/m^3 increment in PM_{10} exposure during 1-y childhood was associated with increases in IL-4 of 0.28% (95%

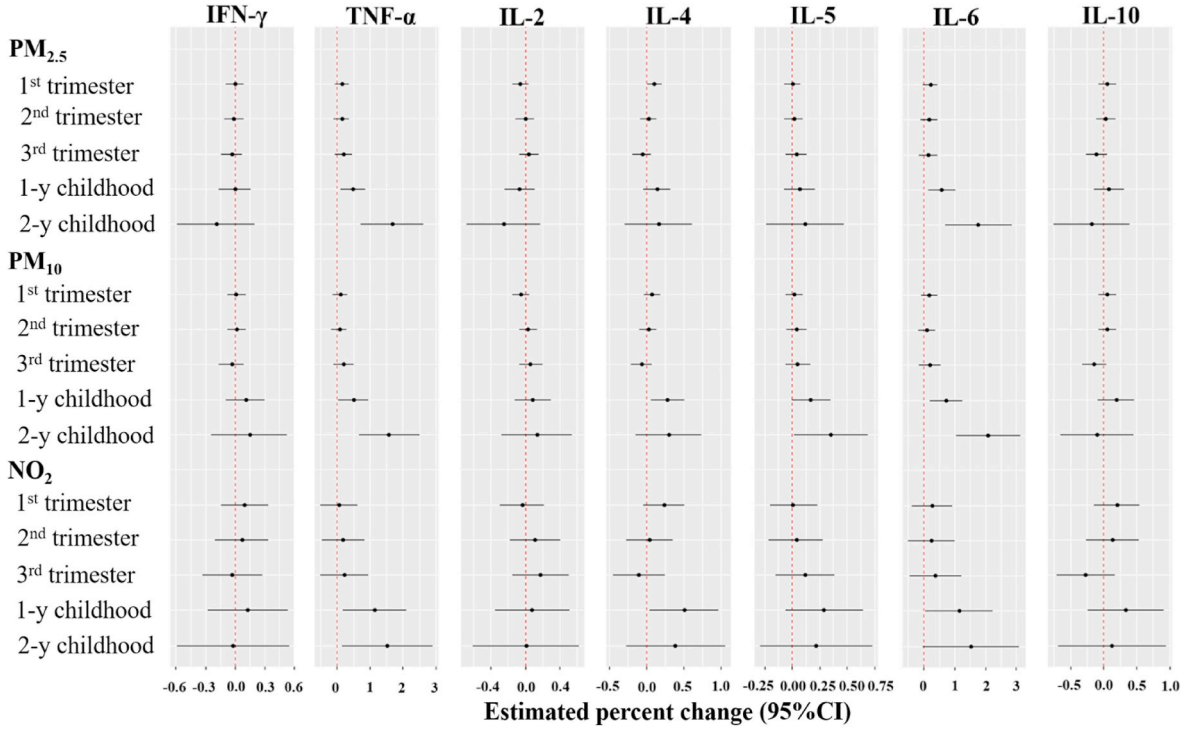

Fig. 3. Adjusted percent changes (95% CIs) in children's plasma cytokines associated with per 10 μg/m^3 incremental change in air pollution exposure at different time windows. All models were adjusted for children age, children sex, season at delivery, maternal age, parity, delivery mode, educational levels, household income, passive smoking during pregnancy, and physical activities during pregnancy.

CI: 0.06%, 0.51%), IL-5 of 0.17% (95% CI: 0, 0.35%), IL-6 of 0.73% (95% CI: 0.21%, 1.27%), and TNF-α of 0.51% (95% CI: 0.05%, 0.97%), and the increasing trends in IL-5, IL-6, and TNF-α persisted during 2-y childhood. Moreover, each 10 μg/m^3 increment in NO_2 exposure during 1-y childhood was related to increases in IL-4 of 0.51% (95% CI: 0.04%, 0.97%), IL-6 of 1.15% (95% CI: 0.06%, 2.24%), and TNF-α of 1.15% (95% CI: 0.18%, 2.12%), and the increasing trend in TNF-α persisted during 2-y childhood. The above associations of prenatal or postnatal air pollution exposures with T lymphocyte subsets and cytokines were not largely changed when additionally adding the adjustment for maternal urinary cadmium or children's plasma cadmium (Tables S4-S5).

3.5. Associations between air pollution exposures and cell-mediated immune markers stratified by children sex

We observed that prenatal or postnatal air pollution exposures showed gender differences for certain cell-mediated immune markers (Fig. 4 and Table S6-S8). For percentage of peripheral blood T lymphocyte subsets, an inverse association of prenatal NO_2 exposure during the first trimester with %CD3$^+$CD4$^+$ cells was observed in boys but not in girls (P for interaction = 0.035), whereas an inverse association with %CD3$^+$CD8$^+$ cells and a positive association with CD4$^+$/CD8$^+$ ratio were observed in girls but not in boys (*P* for interaction = 0.024 and 0.005, respectively). Moreover, inverse associations between postnatal exposure to NO_2 during 2-y childhood and %CD3$^+$CD4$^+$ cells and CD3$^+$CD4$^+$ cells were observed in girls but not in boys (*P* for interaction = 0.019 and 0.010, respectively). We also observed an inverse association of prenatal PM_{10} exposure during the third trimester with CD4$^+$/CD8$^+$ ratio in girls but not in boys (P for interaction = 0.029), whereas a positive association with %CD3$^+$CD4$^+$ cells in boys but not in girls (*P* for interaction = 0.031). For plasma cytokines, we found a positive association of postnatal $PM_{2.5}$ exposure during 2-y childhood with IL-6 in boys but not in girls (*P* for interaction = 0.045), whereas an inverse association with IL-10 among girls but not among boys (*P* for interaction = 0.038).

4. Discussion

In this cohort study of 391 mother-child pairs, we examined the impacts of prenatal and postnatal air pollution exposures on cell-mediated immune responses as reflected by T lymphocyte subsets and cytokines in preschoolers. Overall, exposure to NO_2, PM_{10}, and $PM_{2.5}$ during late pregnancy were positively related to %CD3$^+$ cells and such exposures during 1-y or 2-y childhood were positively associated with plasma cytokines (e.g., IL-6, IL-5, IL-4, and TNF-α). However, exposure to NO_2, PM_{10}, and $PM_{2.5}$ during early pregnancy was associated with decreased %CD3$^+$ cells and %CD3$^+$CD8$^+$ cells. Stratification analyses by child gender showed the sex-specific associations of prenatal or postnatal air pollution exposures with markers of cellular immune responses.

T-lymphocytes play an important role in the cell-mediated immune function (Janeway and Paul, 1976). Experimental studies have shown that air pollutants exposures can alter cell-mediated immune function (Hong et al., 2013; Manners et al., 2014; Chen et al., 2018a). Several cross-sectional studies have also examined the effects of exposure to air pollution on T lymphocyte subsets among adults, with inconsistent results (Salvi et al., 1999; Williams et al., 2009; Pope et al., 2016; Gao et al., 2019). Only a few studies have examined the associations between prenatal air pollution exposures and T lymphocyte subsets in newborns (Hertz-Picciotto et al., 2002; Hertz-Picciotto et al., 2005; Herr et al., 2010; Baïz et al., 2011). Herr et al. (2010) reported that maternal exposure to $PM_{2.5}$ during early pregnancy was related to increases in %CD3$^+$ cells and %CD3$^+$ CD4$^+$ cells, while such exposure during late pregnancy was related to decreases in %CD3$^+$ cells, %CD3$^+$CD8$^+$ cells, and %CD3$^+$CD4$^+$ cells in cord blood of newborns. Baïz et al. (2011) observed that maternal PM_{10} exposure during early pregnancy was

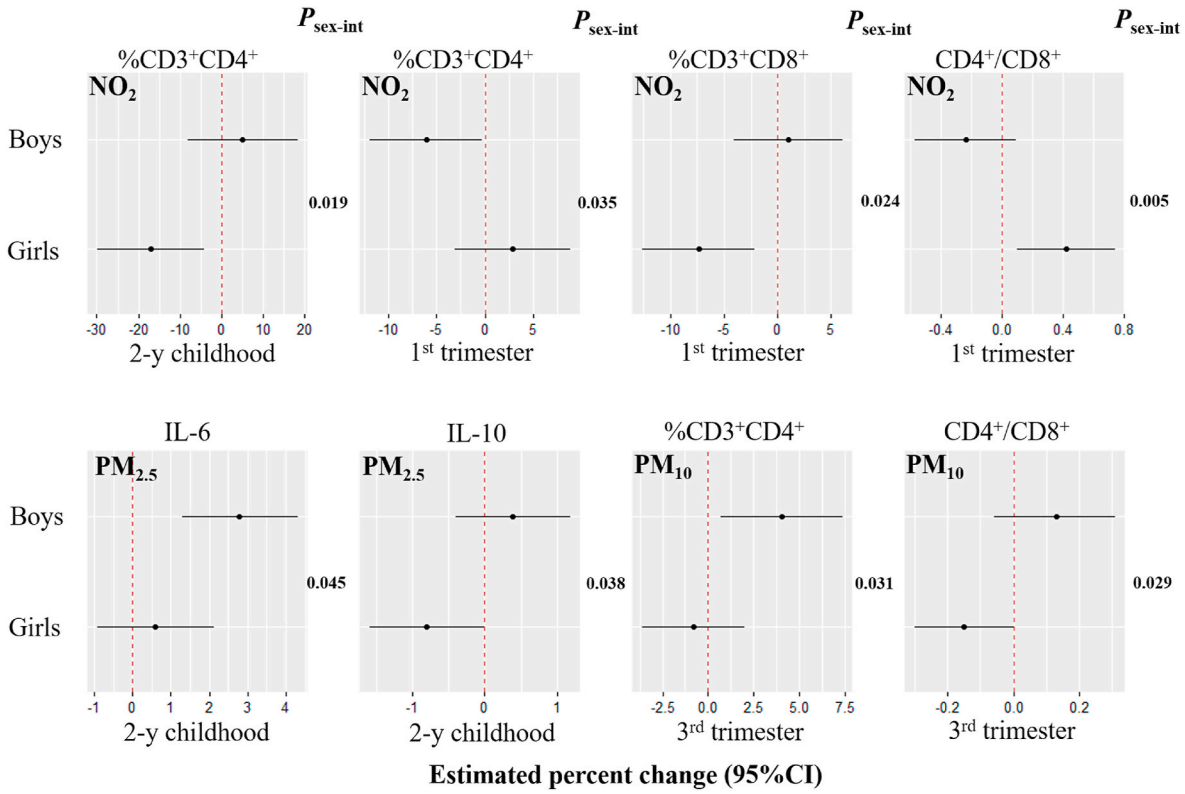

Fig. 4. Adjusted percent changes (95% CIs) in children's percentage of peripheral blood T lymphocyte subsets and plasma cytokines associated with per 10 μg/m^3 incremental change in air pollution exposure stratified by children sex at different time windows. All the models were adjusted for children age, season at delivery, maternal age, parity, delivery mode, educational levels, household income, passive smoking during pregnancy, and physical activities during pregnancy.

associated with decreased %$CD3^+$ cells, whereas such exposures during pre-pregnancy and whole pregnancy were related to increased %$CD3^+CD8^+$ cells in newborn's cord blood. Studies have shown that there are differences in T lymphocyte subsets between newborns and children (Schultz et al., 2000; Gasparoni et al., 2003). To our knowledge, no studies to date have evaluated the effects of prenatal exposure to air pollution on T lymphocyte subsets in children. In this study, we found that maternal exposures to NO_2, PM_{10}, and $PM_{2.5}$ during early pregnancy were negatively associated with %$CD3^+$ cells, %$CD3^+CD8^+$ cells, and %$CD3^+CD4^+$ cells, while exposures to such air pollutants during late pregnancy were positively related to %$CD3^+$ cells.

Cytokines can be produced by innate immune cells (e.g., macrophages and monocytes) and adaptive immune cells (e.g., T-lymphocytes and B-lymphocytes) (Duramad et al., 2007; Bernink et al., 2013; van der Veen et al., 1999). Few epidemiological studies have investigated the associations of postnatal air pollution exposures with cytokines in children (Calderón-Garcidueñas et al., 2013; Klümper et al., 2015; Gruzieva et al., 2017). In Mexico City, compared with those in low-polluted areas of air pollution, children in highly polluted areas had higher levels of IL-6 (Calderon-Garciduenas et al., 2013). Similarly, Klumper et al. (2015) observed that NO_2 exposure was associated with increased TNF-α and IL-6 in asthmatic 6-y-old children. More recently, Gruzieva et al. (2017) observed that NO_2 exposure during infancy in relation to increased IL-6 among 8-y-old children. Our findings showed that air pollution exposures during the first two postnatal years were related to increases in IL-6, IL-5, IL-4, and TNF-α among pre-school children, which were in line with those studies.

We found that air pollution exposures during late pregnancy and the first two years were related to increased T lymphocyte subsets and cytokines, while these exposures during early pregnancy were negatively associated with T lymphocyte subsets. Our findings indicated that air pollution exposures at specific time points may differentially alter cellular immune responses, which may be explained by the varying levels of air pollutants and immune system development. The exposure levels of air pollutants during early trimester in our study were higher than those during late trimester and the first two years, which were primarily attributable to increasingly stringent clean air policy in China and seasonal variation in air pollution (Liao et al., 2018; Zhang et al., 2019). Moreover, the production of T lymphocyte subsets seeded by thymus begins from 10 to 16 weeks of pregnancy (Dietert et al., 2000) and their development may be inhibited by higher air pollution exposures during early pregnancy. After a series of maturational processes, T lymphocyte subsets are well-established during late pregnancy and begin to be differentiated into Th1 and Th2 during the first years of life after encountering specific antigen (West, 2002; Olin et al., 2018). During these periods, the cell-mediated immune responses may be induced by air pollution exposures.

The mechanisms underlying the associations of prenatal or postnatal exposure to air pollution with cell-mediated immune functions remain unknown, and epigenetic changes and oxidative stress have been suggested as potential pathways (Wang et al., 2013). Some studies have provided evidence demonstrating that exposure to air pollution during the early life results in modulation of T cell immune markers by inducing epigenetic alterations including microRNA expression (Chen et al., 2018b), histone modifications, and DNA methylation (Baccarelli and Bollati, 2009). In addition, prenatal exposure to air pollution may induce maternal proinflammatory cytokines production and oxidative stress, resulting in placental dysfunction and enhancing fetal oxidative stress, which in turn influences fetal immune system development and maturation (Yoshida et al., 2012; Wang et al., 2013). Postnatal exposure to air pollution may directly induce oxidative stress and produce the cytokines for activation of Th1 and Th2 cells (Holladay and Smialowicz, 2000).

Our findings also showed the sex-specific associations of prenatal or postnatal air pollution exposures with cellular immune responses, which were based on the specific air pollutants and cellular immune indicators. In support of our findings, a toxicological study found that maternal exposure to PM decreased thymic $CD4^+CD25^+$ cells in female offspring but decreased splenic $CD3^+CD8^+$ and $CD3^+CD4^+$ cells in male offspring (Chen et al., 2018a). Moreover, an epidemiological study indicated that females were more susceptible to air pollutant exposures among children with an allergic predisposition, whereas children without an allergic predisposition showed the opposite pattern (Dong et al., 2011). The biological mechanisms underlying the sex differences are not completely understood but are likely to be involved in sex hormones and lung growth. Males have smaller lung size and higher airway resistance than females during the first 2 years of life (Gehring et al., 2002), and thus the boys may be more vulnerable to postnatal air pollution exposures. Moreover, the antioxidant abilities of sex hormones in females may alleviate the adverse effects of postnatal air pollution exposures on cell-mediated immune responses (Bellanti et al., 2013). Further studies are required to examine the sex differences and investigate the mechanisms of action.

The main strengths of the present study were a prospective design, detailed confounding factors, and multiple markers of cell-mediated immune function including T lymphocyte subsets and cytokines. However, some limitations of the present study were needed to be mentioned. First, the single measurement of cell-mediated immune markers at pre-school age only represented a snapshot in childhood, which limited us to evaluate immune response dynamics throughout the entire pregnancy and childhood period. Second, the strong correlations among air pollutants (NO_2, PM_{10}, and $PM_{2.5}$) and a lack of data on other pollutants (e.g., sulfur dioxide and ozone) limited the interpretation of estimates from the mutually adjusted models. Third, the precision and power of our study were limited as a result of the small sample size, and thus the results observed in this study should be interpreted with caution. Fourth, other exposure sources at work and commute and some potential confounders (e.g., infections, diagnoses of asthma, and dietary) were not considered, which may bias the observed findings.

5. Conclusion

In a cohort study of 391 Chinese mother-child pairs, we found that air pollution exposures during late pregnancy and the first two years were positively related to T lymphocyte subsets and cytokines, while these exposures during early pregnancy were inversely associated with T lymphocyte subsets. These observed effects appeared to be sex-specific. Our findings suggest that air pollution exposures during the different critical windows of early life may differentially induce cellular immune responses. Given that the altered cell-mediated immune responses are likely to be of key importance for the development of immune-mediated diseases, further research on the large-scale population and repeated immune markers is warranted to confirm our results.

Credit author statement

Yan-Ling Deng: Conceptualization, Formal analysis, Methodology, Software, Visualization, Data curation, Writing – original draft, writing-review and editing. Jia-Qiang Liao: Methodology, Supervision, writing-review and editing. Bin Zhou: Investigation, Methodology, Supervision, writing-review and editing. Wen-Xin Zhang: Investigation, Methodology. Chong Liu, Xiao-Qiong Yuan, Pan-Pan Chen, Yu Miao, Qiong Luo, Fei-Peng Cui, and Min Zhang: Investigation. Sheng-Zhi Sun and Tong-Zhang Zheng: Writing -review & editing. Wei Xia, Yuan-Yuan Li, and Shun-Qing Xu: Conceptualization, Resources. Qiang Zeng: Project administration, Conceptualization, Methodology, Supervision, Resources, writing -review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131963.

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