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Traffic-related air pollution in utero modifies cytokine responses to stimuli of umbilical cord blood cells: a cohort study

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ABSTRACT

Objective: To examine the associations between in utero exposure to traffic-related air pollutants (TRAP) and cytokine responses to stimuli in newborns.

Methods: Luminex technology was used to assess cytokine responses in umbilical cord blood of 235 newborns of the NELA cohort. Samples were cultured with mitogens, pathogen associated with molecular patterns (PAMPs) stimuli and common environmental allergens. Dispersion/chemical transport modelling was used to estimate in utero residential exposures to traffic-related nitrogen dioxide (NO_2), particulate matter ($PM_{2.5}$ and PM_{10}) and ozone (O_3). Multivariable linear regression models were fitted.

Results: Per 10 μg/m^3 increase of NO_2, IL-6 increased in response to mitogens Concanavalin A (12.5 %, 95 % CI: 3.6, 21.4) and Phytohemagglutinin (PHA) (14.9 %, 95 % CI: 7.3, 22.5); to PAMPs Lipopolysaccharide (LPS) (11.6 %, 95 % CI: 3.8, 19.5), Peptidoglycan (PG) (12.0 %, 95 % CI: 4.1, 20.0) and pI:C (13.0 %, 95 % CI: 4.9, 21.2); and to allergens Der pT (11.6 %, 95 % CI: 3.4, 19.9) and olive extract (9.7 %, 95 % CI: 0.4, 19.0). IL-6 response to PHA also increased in relation to PM (19.0 % per 5 μg/m^3 increase in $PM_{2.5}$, 95 % CI: 5.5, 32.5; and 20.2 % per 10 μg/m^3 increase in PM_{10}, 95 % CI: 6.0, 34.4). Per 10 μg/m^3 increase of NO_2, IFN-α responses to PHA and PG increased by 7.5 % (95 % CI: 0.6, 14.5) and 7.6 % (95 % CI: 0.1, 15.1), respectively. NO_2 was also associated with an increased Th1-related IFN-γ response to Concanavalin A (7.5 % per 10 μg/m^3 increase, 95 % CI: 0.1, 14.9) and decreased Th2-related IL-5 response to PAMPs PG (−6.7 %, 95 % CI: −12.8, −0.7) and pI:C (−7.6 %, 95 % CI: −14.2, −0.9).

Conclusion: Prenatal exposure to TRAP may promote higher proinflammatory and Th1-related and lower Th2-related cytokine responses to stimuli in the offspring.

1. Introduction

Air pollution has become the second most important environmental risk factor of non-communicable diseases (NCDs) worldwide, after tobacco smoking (Prüss-Ustün et al., 2019), with traffic as the main source for populations living in urban areas (Matz et al., 2019). Exposure to outdoor air pollution has been associated with the onset and progression

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Abbreviations used:

CBMC	cord blood mononuclear cells
Con A	concanavalin A
CpG-ODN	CpG-oligodeoxynucleotide.
Der pT	Dermatofagoides pteronyssinus
IFN	interferon
IL:	interleukin
LPS	lipopolysaccharide.
PAMPs	pathogen-associated molecular patterns
PHA	phytohemagglutinin
pI	C: Polyinosinic:polycytidylic acid
PG	peptidoglycan
PM	particulate matter
Th1	type 1 T helper cells
Th17	type 17 T helper cells
Th2	type 2 T helper cells
TNF	tumor necrosis factor
TRAP	traffic-related air pollution
Treg	regulatory T cells

of respiratory and allergic diseases. Special attention is focused on life periods of higher vulnerability, including the fetal and the early postnatal periods, when long-lasting harmful effects on developmental respiratory and immune systems might be programmed (Hehua et al., 2017; Yang et al., 2019; Hua et al., 2023; García-Serna et al., 2021a; Johnson et al., 2021; Hazlehurst et al., 2021; Wright et al., 2021). The proposed underlying mechanisms include oxidative stress, epigenetic changes, tissue damage and disturbances in the developing immune system, including perturbations in airway inflammatory responses, immune cell distributions and cytokine profiles (Glencross et al., 2020; Murrison et al., 2019; García-Serna et al., 2021b, 2022; Tingskov Pedersen et al., 2023).

To date, few epidemiological studies have investigated whether prenatal exposure to traffic-related pollutants disturbs offspring cytokine profiles at birth (García-Serna et al., 2022; Latzin et al., 2011; Ashley-Martin et al., 2016; Friedman et al., 2021). Exposure to PM_{10} during the third trimester of gestation was associated with increased IL-1β concentrations and exposure to PM_{10} 3 days before delivery associated with decreased IL-10 levels in cord blood of newborns (Latzin et al., 2011). Friedman et al. examined the association between maternal exposure to PM_{10} and O_3 during pregnancy and cytokine concentrations in cord blood collected at birth (Friedman et al., 2021), showing that newborns exposed to higher $PM_{2.5}$ concentrations during the entire pregnancy had decreased concentrations of TNF-α in cord blood (Friedman et al., 2021). Moreover, exposure to O_3 during mid-pregnancy was associated with increased IL-6 and IL-8 concentrations in cord blood, while exposure to O_3 during the last month of pregnancy was associated with decreased IL-6 concentrations in cord blood of newborns (Friedman et al., 2021). Finally, in a previous study we have found that prenatal exposure to traffic-related NO_2 increased the detection of proinflammatory cytokines (IL-1β and IL-6) and Treg-related cytokine IL-10 in cord blood of newborns, and prenatal exposure to traffic-related O_3 increased the detection of IL-13 (García-Serna et al., 2022). Moreover, other studies have also investigated changes in cytokine profiles of children in relation to exposure to air pollutants in early postnatal life (Deng et al., 2022; Calderón-Garcidueñas et al., 2013; Gruzieva et al., 2017). Exposures to NO_2 and $PM_{2.5}$ air pollutants during the first two years of age have been related to increased levels of IL-4, IL-5, IL-6, and TNF-α in children aged 3 years (Deng et al., 2022). In the same way, children living in a highly polluted Mexican area and 8-y-old children exposed to NO_2 during infancy showed increased IL-6 levels in blood (Calderón-Garcidueñas et al., 2013; Gruzieva et al., 2017).

In addition, to our knowledge only one previous study has investigated the effects of prenatal exposure to air pollutants on cytokine responses to stimuli in cord blood of newborns (Hahn et al., 2021). The study, conducted within the Project Viva cohort, investigated the responses of cord blood mononuclear cells (CBMC) to phytohemagglutinin (PHA), cockroach extract (Bla g 2) and house dust mite extract (Der f 1), and analyzed the associations between black carbon and $PM_{2.5}$ exposures across pregnancy and IL-6, IL-10 and TNF-α responses to these stimuli. Results showed that responses to stimuli of both pro-inflammatory cytokines (i.e. IL-6 and TNF- α) and anti-inflammatory cytokine IL-10 in cord blood were lower in relation to higher prenatal $PM_{2.5}$ levels (Hahn et al., 2021). Unravelling comprehensive patterns at a population level of human cytokine responses to prenatal environmental challenges like traffic-related air pollution (TRAP) is required to advance our understanding of the developmental influences shaping these complex immune responses that play a crucial role as immune mechanisms of the susceptibility to respiratory infection, allergic manifestations and asthma pathogenesis during childhood.

Here in, the aim of this study was to investigate the impact of traffic-related air pollution exposure during pregnancy on offspring cytokine responses in umbilical cord blood of newborns to a battery of common different stimuli, including mitogens, pathogen associated with molecular patterns (PAMPs) and common environmental allergens. In addition, trimester-specific exposures were also evaluated to identify critical windows of higher susceptibility across pregnancy.

2. Material and methods

2.1. Study population

The Nutrition in Early Life and Asthma (NELA) study (www.nela.imib.es) is a longitudinal prospective population-based birth cohort set up in Spain whose main aim is to unravel the developmental origins and mechanisms of asthma and allergy. Detailed information on the study design protocol, recruiting methods and data collection has been previously described (Morales et al., 2022). Briefly, from March 2015 to April 2018, a total of 738 pregnant women were recruited at the time of the follow-up visit for routine fetal anatomy scan at 20 weeks of gestation. The inclusion criteria were: women from Health Area I and certain districts of Health Areas VI and VII of the Region of Murcia; planning to live in the area of study during at least 2 years; intention to give birth at the reference hospital; Spanish Caucasian origin; 18–45 years of age; singleton pregnancy; non-assisted conception; and normal echography at 20 weeks of gestation (no major malformations). The exclusion criteria included: an existing chronic disease; pregnancy complications (except gestational diabetes and hypertensive disorders); and not intending to deliver in the reference hospital. Overall, 720 mother-child pairs were followed until birth and cord blood samples were collected in 390 newborns. Cytokine responses to stimuli in umbilical cord blood were assessed in 235 newborns from samples processed within 48 h after birth (Supplementary Fig. S1). There were no differences between them and the remaining participants in a broad range of main characteristics, except that included parents were slightly older and newborns had a slightly longer gestational age (Supplementary Table 1). The study protocol was reviewed and approved by the Ethics Committee of the Virgen de la Arrixaca University Hospital in accordance with the guidelines of The Declaration of Helsinki. Written informed consents were obtained from parents at recruitment.

2.2. Outdoor air pollutants exposure assessment

To assess prenatal exposure to air pollutants, residential address of mothers during pregnancy was geo-codified using Geographic Information System (GIS). Dispersion/chemical transport modelling was applied to estimate individual exposure to air pollutants as previously

described (García-Serna et al., 2021b; Morales et al., 2022). Briefly, the Advanced Weather Research and Forecasting (WRF-ARW) model v3.9.1 + CHIMERE chemistry transport model (CTM) modelling system was coupled off-line for estimating concentrations of outdoor air pollutant in the NELA geographical area (resolution was 0.5 km). Residential long (the entire pregnancy and trimester-specific) and short-term (15-days before delivery) traffic-related exposures were estimated for nitrogen dioxide (NO_2), particulate-matter less than 2.5 μm in diameter ($PM_{2.5}$), particulate-matter less than 10 μm in diameter (PM_{10}) and ozone (O_3).

2.3. Cytokine responses to stimuli in umbilical cord blood of newborns

Venous umbilical cord blood samples were collected at birth in heparinized tubes kept at room temperature and processed within 48 h (Moldenhauer et al., 2007; López et al., 2014; Garcia-Serna et al., 2023). Samples were diluted 1:7 with RPMI 1640 heparinized medium and cultured in presence of different stimuli: mitogens (Concanavalin A (Con A) and Phytohemagglutinin (PHA)); PAMPs (Lipopolysaccharide (LPS), Peptidoglycan (PG), Polyinosinic-polycytidylic acid (pI:C), and cytosine-phosphorothioateguanine oligodeoxynucleotides (CpG-ODN)); and common environmental allergen extracts including the house dust mite *Dermatophagoides pteronyssinus* 1 (Der pT) and the *Olea europaea* pollen extract (Supplementary Table 2). As previously described, supernatants were collected after 7 days of culture (Garcia-Serna et al., 2023; Ter Horst et al., 2016; Bakker et al., 2018) aliquoted and frozen at −80 °C. Luminex technology was used to measure cytokine concentrations using the Human Cytokine Multiplex-Assay-Kit (ThermoFisher, Viena, Austria) according to the manufacturer's instructions. A wide panel of cytokines was designed based on immune system responses: inflammatory innate response (IFN-α, IL1-β, IL-6 and TNF-α), Th1-related (IFN-γ and IL-2), Th2-related (IL-4, IL-5 and IL-13), Th17-related (IL-17 and IL-23), and Treg-related cytokine IL-10. In every experiment we included an internal control consisting of a volume of supernatants obtained after incubating human blood cells with LPS for 7 days. The supernatants were collected and aliquoted in several vials that were kept at −80 °C until their use as internal controls.

2.4. Other variables

Face-to-face questionnaires administered during pregnancy obtained information about maternal and paternal age; maternal and paternal educational level (incomplete secondary or less, complete secondary, and university); parental social class (defined as maternal or paternal occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system: I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled; and unemployed) (Domingo-Salvany et al., 2000); maternal and paternal reported history of asthma (yes/no) and atopy (yes/no); parity (0, nulliparous; vs. 1 or more, non-nulliparous); maternal pre-pregnancy body mass index (BMI) based on height and pre-pregnancy self-reported weight (kg/m2) (categorized as normal BMI <25, overweight 25 < BMI <30, and obesity BMI ≥30); maternal smoking during pregnancy and paternal smoking (yes/no); the area of residency (urban, residential, and rural); pets at home (yes/no); and maternal contact with farming animals during pregnancy (yes/no). Information related to child's sex, birthweight (g); gestational age (weeks); mode of delivery (vaginal non-instrumental, vaginal instrumental, and cesarean section); fever (yes/no) and use of antibiotics during labor (yes/no) was obtained from clinical records. Season of birth (spring, March–May; summer, June–August; fall, September–November; and winter, December–February) was also considered.

2.5. Statistical analysis

Descriptive analyses were performed using Kruskal-Wallis test for continuous variables and Chi-2 test for categorical variables. The Spearman's rank correlation coefficient was computed to evaluate correlations between TRAPs. Cytokine concentration values out of range of detection were imputed using multivariable multiple imputation methods conditioning the imputation to the range (0, LOD) and (>ULOD) (Uh et al., 2008). Multiple imputations of non-detected values were performed using an interval regression imputation method and chained equations (van Buuren et al., 1999; Royston, 2007). Twenty completed data sets were generated and analyzed separately, and the results were combined using Rubin's rules (Little and Rubin, 2014).

We used separate multivariable linear regression models to analyze associations between exposure to TRAP during pregnancy with each cytokine-stimulus response combination. Cytokine concentrations were log transformed and values below detection limits were imputed. Coefficients derived from linear regression represented percentage of change in concentrations and estimators were reported as coefficients in percentage (95 % confidence intervals, CI). Associations between potential confounders and air pollutants were tested using Chi-2 or Kruskal-Wallis tests to compare covariate distributions across levels of categorical air pollution variables or to compare means for continuous air pollution variables across levels of each covariate, and those covariates which showed at least marginally significant association ($p < 0.1$) or modified the regression coefficient of air pollutants by at least 5 % were included. Thus, final models were adjusted by maternal pre-pregnancy BMI, parental social class, maternal atopy, father smoking, season of birth, newborn's sex, gestational age, and birthweight. Further adjustment by the baseline concentration of the cytokine did not materially change the estimates (results not shown). To evaluate the effects of traffic-related pollutant mixture we applied Bayesian kernel machine regression (BKMR) to visualize the exposure-response and verify assumptions using the R package *bkmr* (Bobb et al., 2015). We report group and conditional posterior inclusion probabilities (PIPs). Group PIP represents the probability that a group of air pollutants contributes to the health outcome, while conditional PIP reflects the probability that a specific air pollutant within that group is important.

Data were analyzed in Stata Software (version 15.1, StataCorp, College Station, Texas, USA) and RStudio (version 1.1.463, RStudio, Boston, Mass).

3. Results

Mean (SD) age of women in the study was 33.3 (4.3) years, 51 % of them were nulliparous and about 60 % had a university education (Supplementary Table 1). Most families were classified in the middle or upper social class (75.7 %) and lived in urban areas (73.6 %). Asthma was reported by 14 % of mothers and 9.4 % of fathers. History of atopy was reported by 45.5 % of mothers and 37.6 % of fathers. Overall, 16.6 % of mothers and 35.3 % of fathers reported active tobacco smoking. Positive strong correlations (Spearman's $\rho > 0.7$) were detected between traffic-related NO_2, PM_{10}, and $PM_{2.5}$. Traffic-related O_3 showed moderate correlation with PM_{10} and $PM_{2.5}$ ($0.53 < p < 0.57$), and traffic-related NO_2 and O_3 levels did not correlate.

Higher exposure to residential traffic-related NO_2 and PM during the entire pregnancy was associated with an increased pro-inflammatory response of stimulated cord blood cells (i.e., IFN-α and IL-6 concentrations) to mitogens, PAMPs and common environmental allergens (Fig. 1A and C). For every 10 μg/m^3 increase in NO_2 exposure, IFN-α responses of stimulated cord blood cells to PHA and PG increased by 7.5 % (95 % CI: 0.6, 14.5) and 7.6 % (95 % CI: 0.1, 15.1), respectively. IFN-α response to PHA also tended to increase in relation to higher prenatal exposure to PM_{10} and $PM_{2.5}$ (Fig. 1A). Exposure to NO_2 during pregnancy was associated with increased IL-6 response of stimulated cord blood cells to mitogens Con A (12.5 % per 10 μg/m^3 increase in NO_2, 95 % CI: 3.6, 21.4) and PHA (14.9 % per 10 μg/m^3 increase in NO_2, 95 % CI: 7.3, 22.5); to PAMPs, including LPS (11.6 % per 10 μg/m^3 increase in NO_2, 95 % CI: 3.8, 19.5), PG (12.0 % per 10 μg/m^3 increase in NO_2, 95 % CI: 4.1, 20.0) and pI:C (13.0 % per 10 μg/m^3 increase in NO_2, 95 % CI:

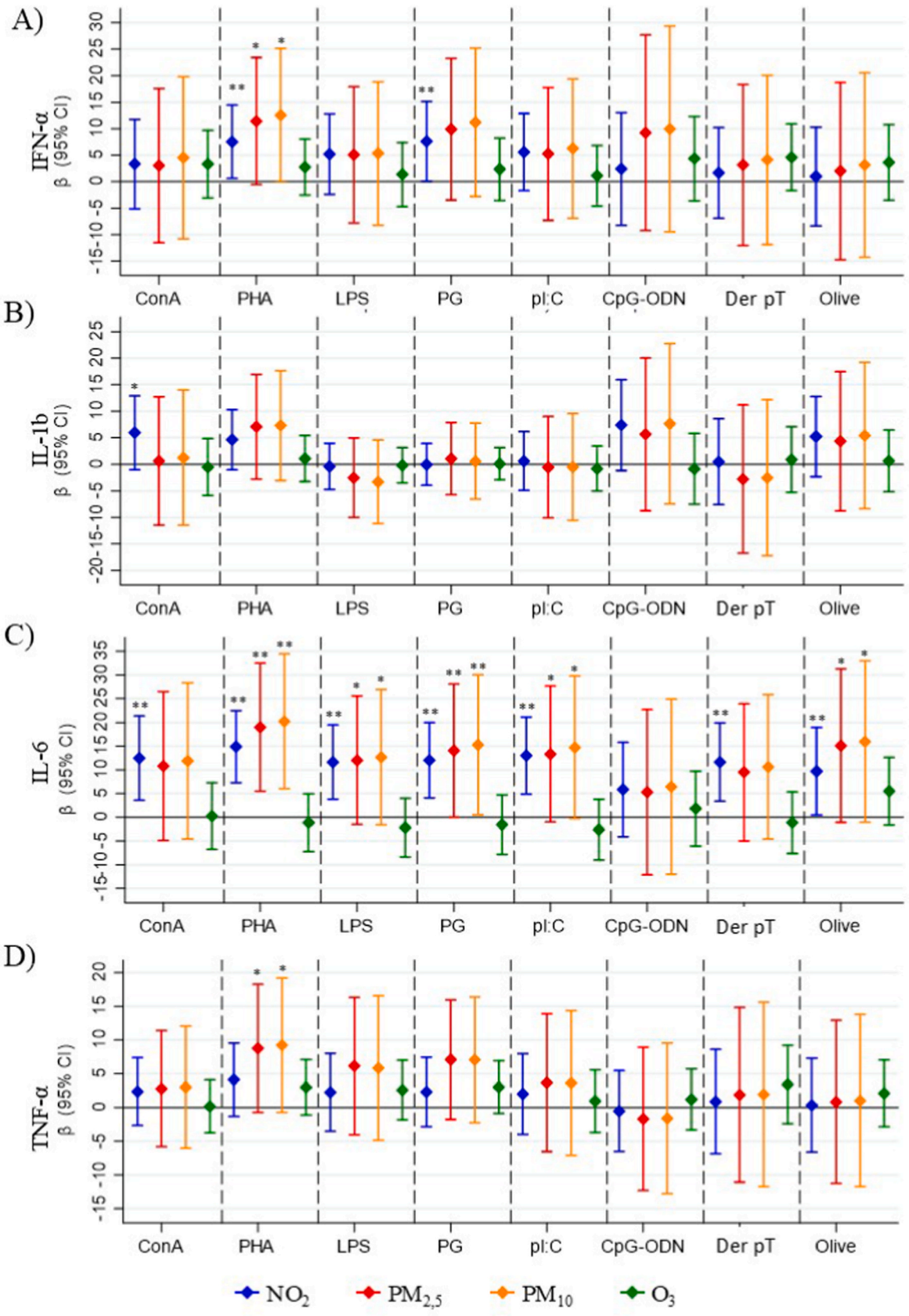

Fig. 1. Percent difference in pro-inflammatory cytokines (i.e., IFN-α, IL-1β, IL-6 and TNF-α) concentrations in cord blood of newborns in response to mitogens (Con A and PHA), PAMPs (LPS, PG, pI:C and CpG-ODN) and allergens (Der pT and olive extract) per 10 μg/m3 increase in NO2, per 10 μg/m3 increase in PM10, per 5 μg/m3 increase in PM2.5 and per 10 μg/m3 increase in O3 during entire pregnancy. Coefficients derived from multivariable linear regression models adjusted for maternal pre-pregnancy BMI, parental social class, maternal atopy, father smoking, season of birth, newborn's sex, gestational age, and birthweight. *p value > 0.05 and <0.1; **p value < 0.05.

4.9, 21.2); and allergens including Der pT (11.6 % per 10 μg/m^3 increase in NO_2, 95 % CI: 3.4, 19.9) and olive extract (9.7 % per 10 μg/m^3 increase in NO_2, 95 % CI: 0.4, 19.0) (Fig. 1C). Moreover, increased IL-6 response to PHA was found in relation to higher prenatal exposure to PM (19.0 % per 5 μg/m^3 increase in $PM_{2.5}$, 95 % CI: 5.5, 32.5; and 20.2 % per 10 μg/m^3 increase in PM_{10}, 95 % CI: 6.0, 34.4). IL-6 response of cord blood cells also tended to increase after stimulation with PAMPs and olive extract among newborns exposed to higher PM concentrations.

Higher prenatal exposure to NO_2 during the entire pregnancy was associated with an increased Th1-related IFN-γ response to mitogens Con A (7.5 % per 10 μg/m^3 increase in NO_2, 95 % CI: 0.1, 14.9) and PHA (8.4 % per 10 μg/m^3 increase in NO_2, 95 % CI: −0.5, 17.2) (Fig. 2A). IL-2 and Th-17 related responses (i.e., IL-17F and IL-23) to stimuli did not differ in relation to prenatal levels of TRAP (Fig. 2B, C and D). Th2-related cytokine responses to stimuli are shown in Fig. 3A–C. Maternal exposure to NO_2 during pregnancy were associated with decreased IL-5 response to bacterial PAMPs and pI:C (Fig. 3B); and prenatal exposures to PM were associated with increased IL-4 response to PHA (Fig. 3A). Th2-related IL-5 response to Der pT extract decreased in newborns exposed to NO_2 (Fig. 3B). Furthermore, Treg-related IL-10 response to Con A and CpG-ODN tended to increase among newborns prenatally exposed to higher levels of NO_2 during the entire pregnancy (Fig. 3D).

Supplementary Tables S3–S5 show associations between short-term exposure to TRAP (i.e. 15 days before delivery) and cytokine responses to stimuli in cord blood. Higher levels of NO_2 were associated with increased production of IL-6, IFN-α, IFN-γ, IL-10 and IL-13 in response to mitogens, as well as with increased production of IL-6 in response to PAMPs and Der pT. Exposure to higher levels of PM was associated with increased production of IL-6 in response to mitogens. IL-5 response to PAMPs and allergen Der pT was reduced in newborns of mothers exposed to higher levels of TRAP.

We fitted the BKMR model to analyze the simultaneous prenatal exposure to the mixture of TRAPs and selected cytokine responses to stimuli at birth (Table 1). BKMR models showed that pro-inflammatory cytokine responses to stimuli in newborns were driven in the mixture by prenatal exposure to NO_2, including IFN-α response to PG (group PIP 0.537), and IL-6 responses to PHA, PG and Der pT (group PIP: 0.836, 0.827 and 0.918, respectively) (Table 1). Similarly, Th1-related IFN-γ response to mitogen Con A was also driven by prenatal NO_2 (group PIP: 0.827). However, IL-6 response to olive extract was driven by prenatal exposure to PM. Linear positive dose-response relationships between exposure to TRAP mixture during pregnancy and cytokine responses to stimuli in newborns were confirmed by BKMR models. In addition, no interaction among TRAP mixture was found (Supplementary Fig. S2).

Stratified analyses by trimester of gestation showed similar associations between prenatal traffic-related NO_2 and PM concentrations and cytokine responses to stimuli in umbilical cord blood across trimesters (Supplementary Tables S6–S13).

4. Discussion

As far as we know, this is the most comprehensive epidemiological study investigating the impact of in utero exposure to TRAP on cytokine responses to a large variety of stimuli in newborns. Single-pollutant models showed that production of pro-inflammatory cytokines (i.e., IL-6 and IFN-α) in cord blood in response to specific stimuli (i.e., mitogens, PAMPs and common allergens) increased among newborns whose mothers were exposed to higher traffic-related NO_2 and PM concentrations during pregnancy. Moreover, newborns exposed to higher levels of traffic-related NO_2 showed an increased Th1-related IFN-γ response to mitogens (i.e. ConA and PHA) and a decreased Th2-related IL-5 response to PAMPs (i.e. bacterial and pI:C). Multi-pollutant BMKR models confirmed the linear associations and identified NO_2 as the main driver among the TRAP mixture for most of the observed associations. In stratified analyses, we did not observe windows of higher susceptibility of prenatal exposure to TRAP on cytokine responses to stimuli at birth.

Our findings are not in accordance with the results reported by the only previous study evaluating neonates' CBMC cytokine responses (i.e. IL-6, IL-10 and TNF-α) to stimuli (i.e. PHA, cockroach and house dust mite extracts) in relation to air pollution across pregnancy (i.e. black carbon and $PM_{2.5}$) (Hahn et al., 2021). Unexpectedly, authors found that responses of pro-inflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine IL-10 were all lower among newborns of mothers exposed to higher levels of $PM_{2.5}$ during pregnancy. However, based on previous knowledge, increased exposure to air pollution during pregnancy would lead to higher concentrations of pro-inflammatory cytokines and lower concentrations of anti-inflammatory cytokines assayed from newborns' cord blood (Latzin et al., 2011; García-Serna et al., 2022).

We found that prenatal exposure to higher TRAP levels may predispose the developing immune system toward pro-inflammatory responses to diverse stimuli. We found higher prenatal concentrations of NO_2 and PM_{10}, to be associated with an increased IL-6 release in cord blood in response to mitogens, PAMPS and common allergens. Previous studies have shown associations between prenatal NO_2 and PM exposure and increased IL-6 levels in cord blood of newborns (García-Serna et al., 2022) and in early childhood (Deng et al., 2022; Calderón-Garcidueñas et al., 2013; Gruzieva et al., 2017). Interestingly, increased IL-6 levels are found in children with viral respiratory tract infections, associated with increased mortality in children younger than 5 years of age with severe pneumonia (Duan et al., 2025). Moreover, IL-6 increase associated with respiratory infections may contribute to development of recurrent wheezing or asthma (Zeng et al., 2011) and impaired lung function (Peters et al., 2016) later in life. In addition, we found in utero exposure to traffic-related NO_2 to be associated with an increased IFN-α response to mitogen PHA and pathogen-associated PG. IFN-α is the principal IFN produced during innate responses to respiratory viruses in PBMCs and has a key role in the initiation of lung inflammation responses (Khaitov et al., 2009). Like this, IFN-α response helps control virus burden but can also cause detrimental immunopathology and contribute to disease severity (Makris et al., 2017). Furthermore, there is now increasing evidence that type I IFNs are not only induced after viral infections but also after infection with bacteria and fungi (Makris et al., 2017). Interestingly, during asthma exacerbations associated with respiratory virus (RV), asthmatic children can induce IFN-α secretion, indicating a hyperactive immune response to repeated respiratory virus infection (Bergauer et al., 2017). In view of our results, we propose that the associations reported by epidemiological studies between prenatal exposure to outdoor air pollutants and higher risk of wheezing, asthma and worse lung function in childhood could be explained by a predisposition of the immune system towards a pro-inflammatory response (i.e., IL-6 and IFN- α) to microbial stimuli due to the detrimental and long-lasting effects of prenatal exposure to air pollutants on the developing immune system. A pro-inflammatory response is the first mechanism removing pathogens, but prolonged or excessive inflammatory environment could lead to damage tissue. Thus, prenatal exposure to higher levels of TRAP may predispose children to a hyperactive immune response to microbial infections and exacerbated airway inflammation leading to higher risk of wheezing, asthma and impaired lung function.

Moreover, our results showed that exposure to prenatal TRAP was related to increased Th1-related IFN-γ and decreased Th2-related IL-5 responses to stimuli, which may confer risk of allergic manifestations and respiratory infection later in life. IFN- γ has a lower specific antiviral activity and presents more immunomodulatory properties in comparison with other type I IFNs. Although growing evidence suggests a protective role for IFN-γ against allergic diseases, conflicting results still regard its involvement in these responses. Some studies have reported that children who eventually develop atopy manifest higher levels of IFN-γ production than their nonatopic counterparts (Smart and Kemp, 2002; Rowe et al., 2004). In the context of allergic diseases, IFN-γ has been suggested to play a central role in the inflammatory process at lesional sites in atopic dermatitis (Grewe et al., 1998) and in airway

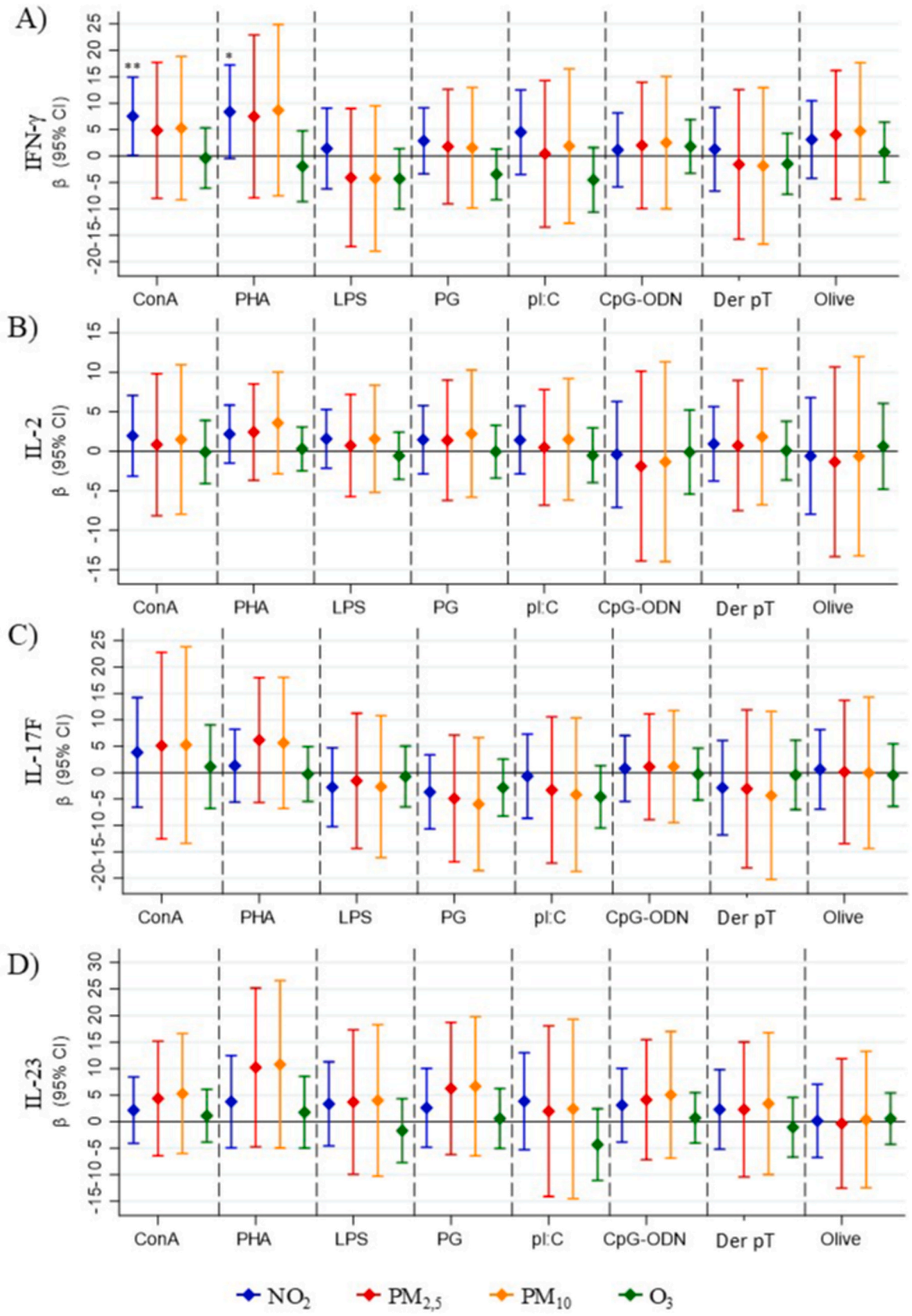

Fig. 2. Percent difference in Th1-related (IFN-γ and IL-2) and Th-17-related (IL-17F and IL-23) concentrations in cord blood of newborns in response to mitogens (Con A and PHA), PAMPs (LPS, PG, pI:C and CpG-ODN) and allergens (Der pT and olive extract) per 10 μg/m3 increase in NO2, per 10 μg/m3 increase in PM10, per 5 μg/m3 increase in PM2.5 and per 10 μg/m3 increase in O3. Coefficients derived from multivariable linear regression models adjusted for maternal pre-pregnancy BMI, parental social class, maternal atopy, father smoking, season of birth, newborn's sex, gestational age, and birthweight. *p value $>$ 0.05 and $<$0.1; **p value $<$ 0.05.

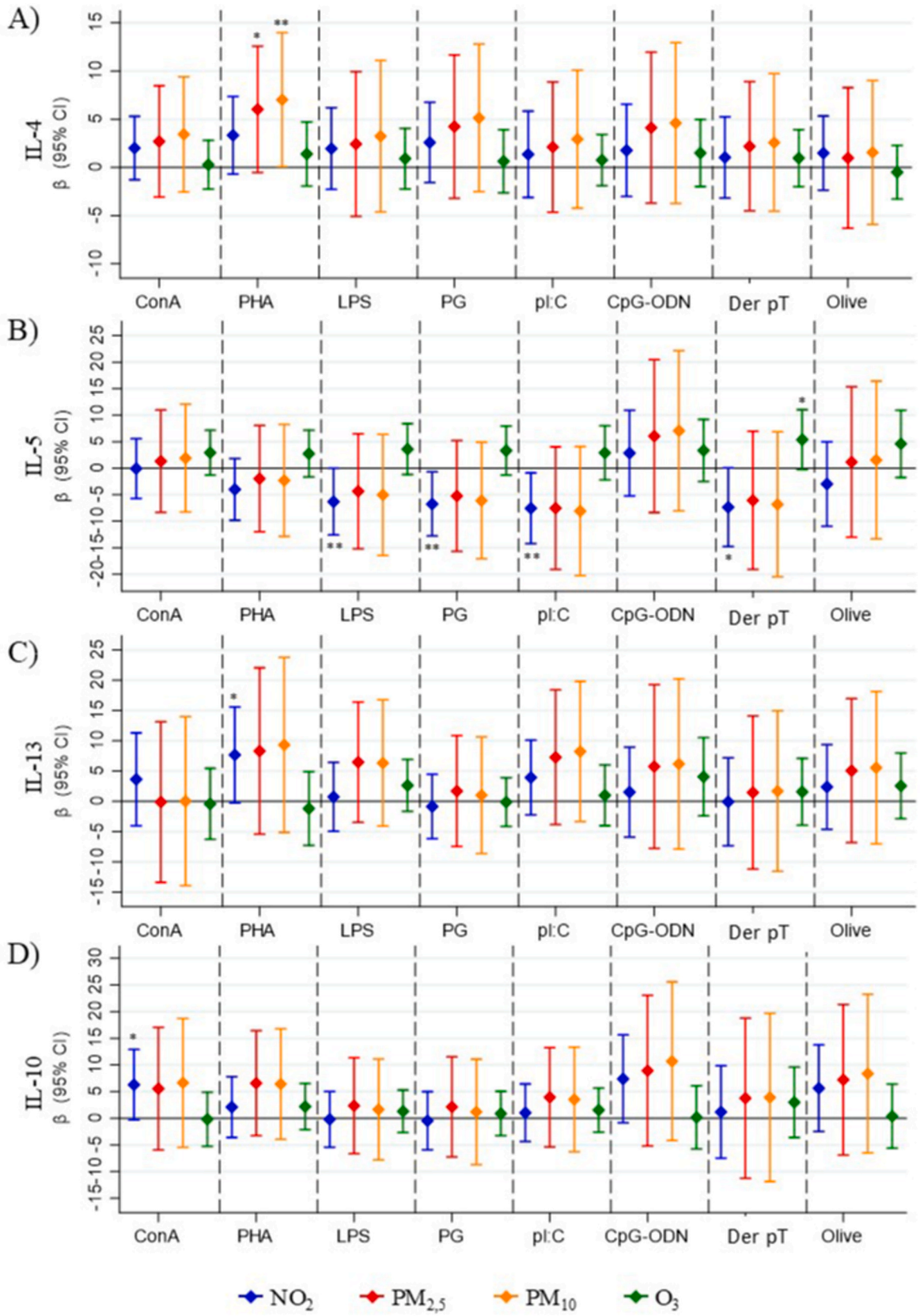

Fig. 3. Percent difference in Th2-related (IL-4, IL-5 and IL-13) and Treg-related (IL-10) concentrations in cord blood of newborns in response to mitogens (Con A and PHA), PAMPs (LPS, PG, pI:C and CpG-ODN) and allergens (Der pT and olive extract) per 10 μg/m3 increase in NO2, per 10 μg/m3 increase in PM10, per 5 μg/m3 increase in PM2.5 and per 10 μg/m3 increase in O3. Coefficients derived from multivariable linear regression models adjusted for maternal pre-pregnancy BMI, parental social class, maternal atopy, father smoking, season of birth, newborn's sex, gestational age, and birthweight. *p value $>$ 0.05 and $<$0.1; **p value $<$ 0.05.

Table 1
Group and conditional posterior inclusion probabilities (PIPs) from BMKR models.

	Group PIP	Conditional PIP
IFN-α response to PHA		
NO	0.581 (±0.106)	
$PM_{2.5}$	0.599 (±0.069)	0.472 (±0.036)
PM_{10}	0.599 (±0.069)	0.528 (±0.036)
O_3	0.370(±0.078)	
IFN-α response to PG		
NO_2	0.537 (±0.086)	
$PM_{2.5}$	0.523 (±0.086)	0.472 (±0.015)
PM_{10}	0.523 (±0.086)	0.528 (±0.015)
O_3	0.315 (±0.097)	
IL-6 response to PHA		
NO_2	0.836 (±0.053)	
$PM_{2.5}$	0.522 (±0.057)	0.448 (±0.016)
PM_{10}	0.522 (±0.057)	0.552 (±0.016)
O_3	0.167 (±0.029)	
IL-6 response to PG		
NO_2	0.827 (±0.063)	
$PM_{2.5}$	0.447 (±0.090)	0.420 (±0.047)
PM_{10}	0.447 (±0.090)	0.580 (±0.047)
O_3	0.150 (±0.029)	
IL-6 response to Der pT		
NO_2	0.918 (±0.031)	
$PM_{2.5}$	0.570 (±0.053)	0.439 (±0.013)
PM_{10}	0.570 (±0.053)	0.561 (±0.013)
O_3	0.240 (±0.041)	
IL-6 response to olive		
NO_2	0.624 (±0.070)	
$PM_{2.5}$	0.737 (±0.043)	0.495 (±0.013)
PM_{10}	0.737 (±0.043)	0.505 (±0.013)
O_3	0.547 (±0.082)	
IFN-γ response to ConA		
NO_2	0.827 (±0.063)	
$PM_{2.5}$	0.447 (±0.090)	0.437 (±0.029)
PM_{10}	0.447 (±0.090)	0.563 (±0.029)
O_3	0.150 (±0.029)	

Group PIP (±SD) indicates the posterior probability of an exposure domain being included in the model; conditional PIP (±SD) indicates the posterior probability of a single exposure within the domain to be included in the model. Models were adjusted for maternal pre-pregnancy BMI, parental social class, maternal atopy, father smoking, season of birth, newborn's sex, gestational age, and birthweight.

inflammation in animal models of asthma (Hessel et al., 1997). Moreover, asthma severity in children has been linked with excessive IFN-γ production, in particular by $CD8^+$ T cells (Magnan et al., 2000) and bronchoalveolar lavage studies suggest preferential recruitment of $CD8^+$ T cells (relative to $CD4^+$ T cells) into the airways during acute asthma exacerbations. Furthermore, exposure to traffic-related NO_2 across pregnancy and short-term exposure before delivery to PM was associated with decreased Th2-related IL-5 response to bacterial PAMPs in newborns, which suggests a suppressed Th2-related immune response and an early immunosuppressive environment at birth. An initial suppressive effect may be inferred as beneficial regarding early asthma symptoms; however, studies on animal models have proposed that an overall adverse effect from combustion-derived air pollutants exposure as attenuated immune response during a period of immune maturation likely predisposes offspring to respiratory infection and leads to an exacerbated allergic response later in life (Saravia et al., 2014; Rychlik et al., 2019).

The main strengths of this study are its population-based and prospective design, and the comprehensive assessment of the impact of traffic-related air pollutant on cytokine responses (i.e., pro-inflammatory, Th1-related, Th2-related and Treg) in umbilical cord blood to a large variety of stimuli. Moreover, study design allowed us to collect detailed information on potential confounders. Another strength was the assessment of outdoor air pollution exposure in utero using WRF-Chem models that provide a high spatial and temporal resolution assessing pollutants not routinely monitored and modelling short- and long-term exposure periods.

Our study has some limitations. The current results are based on a subsample of the NELA birth cohort. Potential differences between included and excluded study participants were controlled in multivariable adjustment analyses to preserve the internal and external validity of the results. Moreover, limited study power may affect the precision of the estimates. Although multiple comparisons may cause type I error, we did not apply false discovery rate (FDR) correction given that variables are correlated to each other and FDR correction (Benjamini–Hochberg procedure) assumes that tests are independent or positively regression dependent. Instead, we applied Bayesian models to deal with air pollutant correlation. In utero exposure to TRAP was based on WRF-Chem models applied to geocoded home address without considering time-activity patterns of pregnant women and assuming that those spend a larger fraction of time in their residence, which could result into exposure misclassification of study participants. Moreover, analyzing high correlated concurrent air pollutants during different time windows across pregnancy makes difficult the identification of independent health effects; however, this does not modify the public health implications of the findings. Information on temperature was not available; however, models were adjusted for season of birth to minimize potential residual confounding. Finally, we did not examine any adverse health outcome in the offspring; however, follow-up has continued for the NELA study, which offers the opportunity to extend these findings to examine associations of immune responses at birth with health outcomes throughout childhood, including respiratory infections, allergic manifestations, asthma and lung function.

In conclusion, in utero exposure to outdoor traffic-related NO_2 and PM, even at concentrations below the limits of standard air quality guidelines, might predispose the developing immune system towards a pro-inflammatory response (i.e. IL-6 and IFN-α) to specific stimuli, including mitogens, pathogens, and common allergens. Moreover, prenatal exposure to traffic related NO_2 disturbed Th1 and Th2-related cytokine responses to pathogens. We propose that observed disturbances on immune system responses to stimuli are likely underlying mechanisms linking the reported associations between prenatal exposure to outdoor air pollutants and higher susceptibility to respiratory infections, asthma, allergic manifestations and impaired lung function during childhood. Further global policies and efforts are still needed to improve air quality to protect the immune system in critical periods of vulnerability during early life development.

CRediT authorship contribution statement

Azahara M. García-Serna: Writing – original draft, Formal analysis, Conceptualization. **Elena Martín-Orozco:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Pedro Jiménez-Guerrero:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Trinidad Hernández-Caselles:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Esther Cantero-Cano:** Writing – review & editing, Data curation. **María del Carmen Elena:** Writing – review & editing, Data curation. **Jesús Soler-Sánchez:** Writing – review & editing, Data curation. **Luis García-Marcos:** Writing – review & editing, Funding acquisition, Conceptualization. **Eva Morales:** Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

All authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2026.123739.

Data availability

Data will be made available on request.

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