

# Exposure to Traffic-Related Air Pollution and Serum Inflammatory Cytokines in Children

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**BACKGROUND:** Long-term exposure to ambient air pollution can lead to adverse health effects in children; however, underlying biological mechanisms are not fully understood.

**OBJECTIVES:** We evaluated the effect of air pollution exposure during different time periods on mRNA expression as well as circulating levels of inflammatory cytokines in children.

**METHODS:** We measured a panel of 10 inflammatory markers in peripheral blood samples from 670 8-y-old children in the Barn/Child, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) birth cohort. Outdoor concentrations of nitrogen dioxide (NO<sub>2</sub>) and particulate matter (PM) with aerodynamic diameter <10 μm (PM<sub>10</sub>) from road traffic were estimated for residential, daycare, and school addresses using dispersion modeling. Time-weighted average exposures during infancy and at biosampling were linked to serum cytokine levels using linear regression analysis. Furthermore, gene expression data from 16-year-olds in BAMSE (*n* = 238) were used to evaluate links between air pollution exposure and expression of genes coding for the studied inflammatory markers.

**RESULTS:** A 10 μg/m<sup>3</sup> increase of NO<sub>2</sub> exposure during infancy was associated with a 13.6% (95% confidence interval (CI): 0.8; 28.1%) increase in interleukin-6 (IL-6) levels, as well as with a 27.8% (95% CI: 4.6, 56.2%) increase in IL-10 levels, the latter limited to children with asthma. However, no clear associations were observed for current exposure. Results were similar using PM<sub>10</sub>, which showed a high correlation with NO<sub>2</sub>. The functional analysis identified several differentially expressed genes in response to air pollution exposure during infancy, including *IL10*, *IL13*, and *TNF*.

**CONCLUSION:** Our results indicate alterations in systemic inflammatory markers in 8-y-old children in relation to early-life exposure to traffic-related air pollution. <https://doi.org/10.1289/EHP460>

## Introduction

Air pollution exposure has been related to an increased risk of respiratory and allergic disorders in children (Gehring et al. 2015; Nordling et al. 2008; Schultz et al. 2016). Although the exact biological mechanisms are not yet understood, antioxidant and inflammatory responses have been suggested among the key pathways (Esposito et al. 2014; Minelli et al. 2011). The link between the local inflammatory process in the airways and systemic inflammation is considered to involve inflammatory markers secreted by alveolar macrophages and bronchial epithelial cells in response to air pollution exposure, which are capable of entering the bloodstream and stimulating acute-phase reactions (Brook et al. 2010). In addition, direct systemic effects by inhaled particulate matter (PM) that translocates from lungs into the vascular tree, inducing the release of proinflammatory mediators have been demonstrated (Nakane 2012). Many studies have reported associations between short-term exposure to air pollution and acute changes in inflammatory markers, such as increase in pulmonary interleukin (IL-6) measured in exhaled breath condensate (Behndig et al. 2006; Kongerud et al. 2006; Thompson

et al. 2010), as well as systemic IL-6 and TNFα levels in adult populations (Tsai et al. 2012), including subgroups with preexisting chronic inflammatory conditions (Rückerl et al. 2007). However, others failed to demonstrate associations with IL-6 (Bräuner et al. 2008; Panasevich et al. 2009; Zurbier et al. 2011) or TNF-α (Bräuner et al. 2008; Larsson et al. 2013). Studies of long-term exposure to air pollution demonstrate mixed effects on blood markers of systemic inflammation (Chuang et al. 2011; Forbes et al. 2009; Mostafavi et al. 2015; Panasevich et al. 2009). Only very few epidemiological studies investigated the association between traffic-related air pollution (TRAP) and cytokine levels in children. Elevated serum levels of IL-6 in relation to long-term air pollution exposure have been reported (Calderon-Garcidueñas et al. 2013; Klümper et al. 2015), but other studies did not find associations with studied inflammatory markers (Armijos et al. 2015; Brown et al. 2012). The inconsistency in findings may be partially attributed to methodological differences (e.g., sample size, model specifications, exposure assessment), as well as the actual levels of exposure to air pollutants.

A few molecular studies of adults showed that genetic variants in the *IL6* gene may modify effects of both long- and short-term air pollution exposure on inflammatory markers (Ljungman et al. 2009; Panasevich et al. 2013). Further mechanistic support comes from *in vitro* studies showing significantly higher time- and dose-dependent expression of the *IL6* gene (Bach et al. 2015; Liu et al. 2014; Shang et al. 2013; Totlandsdal et al. 2010) in cultured human lung epithelial cells following fine PM exposure. Furthermore, associations were also found with indoor particulate exposure and the level of *IL6* expression in sputum of healthy children (Nazariah et al. 2013). These effects have largely been investigated in studies of short-term exposure to air pollution, there is a lack of mechanistic studies investigating long-term effects. Further investigation of changes in blood inflammation parameters as well as their functional importance is thus needed to elucidate the pathways of disease development caused by

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long-term air pollution exposure as well as to identify appropriate biomarkers.

The aim of the present study was to investigate the association of serum inflammatory markers in school-age children with exposure to NO<sub>2</sub>, an indicator of combustion-related ambient air pollution, as well as PM<sub>10</sub>, mostly representing particles mechanically generated from road dust, during different time periods. In addition, we aimed to assess the influence of genetic polymorphisms on the cytokine response to air pollution and to explore potential gene–environment interactions. Finally, we evaluated whether air pollution exposure influenced the expression of genes encoding the studied cytokines, as well as the role of genetic polymorphisms on the mRNA expression response to air pollution exposure.

## Methods

### Study Population

The Barn/Child, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) study is a population-based birth cohort in which infants were recruited at birth and prospectively followed during childhood and adolescence. A total of 4,089 children born in Stockholm, Sweden, between 1994 and 1996 were included (Wickman et al. 2002). At a median infant age of 2 mo, parents completed a baseline questionnaire that assessed environmental exposures, parental smoking habits, residential characteristics, lifestyle, and parental allergies. When children were approximately 1, 2, 4, 8, 12 and 16 y or age, parents completed questionnaires focusing on symptoms of asthma, rhinitis, and eczema in their children and then-current parental smoking habits (Thacher et al. 2014). Response rates were 96%, 94%, 91%, 84%, 82%, and 78%, respectively. Blood samples were collected at ages 4, 8, and 16 y old from 2,605 (63.7%), 2,470 (60.4%) and 2,547 (62.2%) children, respectively.

For the current study, cytokine levels were measured in a sample of 670 subjects, of whom 473 children were selected based on the availability of other biological data, such as GWAS (genome-wide association study) genotypes. Of these, children with a doctor's diagnosis (DD) of asthma ever up to 8 years old were selected as asthma cases ( $n = 237$ ) and an equivalent number of children with no history of asthma or other allergic diseases (e.g., rhinitis and eczema) served as controls ( $n = 236$ ) (Melén et al. 2013). In addition, about 200 children were selected based on contrasts in air pollution exposure levels during infancy and current exposure at 8 years of age: 105 children with high exposure during infancy (PM<sub>10</sub> > 75th percentile of the exposure range in the cohort) and lower current exposure level at 8 y of age (PM<sub>10</sub> < 25th percentile), and 92 children with lower exposure during infancy and high current exposure at 8 y of age. Exposure during infancy was defined as the average concentration of the respective pollutant at the residential address during the first year of life (from birth to the first-year birthday) using modeled annual levels of pollutants. Current exposure at 8 y of age corresponds to average concentration of air the pollutant during the last 12 mo prior to biosampling at the 8-y-follow-up. Age- and municipality-specific data on the time children were at child care facilities and schools were used to estimate the number of hours spent at home, child care facilities, and schools. Time-weighted average exposure to NO<sub>2</sub> and PM<sub>10</sub>, respectively, was estimated for each child, depending on the duration of time spent at the different locations. We took changes in residence into account and, in case parents provided two home addresses for the same period (i.e., related to divorce), the child was assumed to have spent 50% of the time at each place. For the first year of life,

only residential addresses were considered because children in Sweden usually do not start daycare before the age of 12 mo.

The Regional Ethical Review Board, Karolinska Institutet, Stockholm, Sweden, approved baseline and all follow-up studies, and the parents of all participating children provided informed consent.

### Air Pollution Exposure Assessment

The methodology for calculating individual long-term exposure to locally emitted traffic-related NO<sub>2</sub> and PM<sub>10</sub> is described in detail elsewhere (Schultz et al. 2016). In brief, the annual mean concentrations of NO<sub>2</sub> and PM<sub>10</sub> were calculated using a Gaussian air quality dispersion model and a wind model, both part of the Airviro Air Quality Management System (<http://airviro.smhi.se>). The calculations were performed on a 25-m resolution grid for addresses in the more densely populated areas of Stockholm County, such as urban areas, and 100-m or a 500-m grid in less densely populated areas. Emission databases for NO<sub>2</sub> were available for the years 1990, 1995, 2000, 2002, 2003, 2004, 2006, 2010, and 2015. To obtain NO<sub>2</sub> concentrations for all years during the period of interest, the model calculations were interpolated. PM<sub>10</sub> model calculations were performed only for the year 2004, when the most complete database was available and applied to all years during the observation period. In addition, a street canyon contribution was added for addresses in the most polluted street segments in the inner city of Stockholm with multistorey houses on both sides, using the Airviro street canyon model (<http://airviro.smhi.se>).

### Outcome Assessment

The levels of inflammatory cytokines IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF- $\alpha$  were measured using electro-chemiluminescence 10-spot MULTISPOT<sup>®</sup> 96 well plates manufactured by Meso Scale Discovery Multi Array (MSD), using MSD 2,400 instrument (Velásquez et al. 2014). The concentrations of serum levels were obtained from the 8-point standard curve, and the units are expressed as picograms per milliliter (pg/ml). For intra-assay variability  $n = 50$ , samples were run in duplicates. All analyses were performed according to the manufacturer's assay protocol.

DNA was extracted from peripheral blood leucocytes. The samples were genotyped using the Illumina Human610-Quad BeadChip (Illumina Inc, San Diego, CA, USA), and the genotyping has been described elsewhere (Moffatt et al. 2010).

Data on mRNA gene expression were available in 238 BAMSE children aged 16 y old, of whom 172 had measured serum inflammatory markers at 8 y of age (Gruzieva et al. 2017). Whole blood was collected in PAXGene tubes, and RNA was extracted using the PAXgene Blood RNA kit (QIAGEN). Assessments of extracted RNA yield and quality were performed with DropSense96 (Trinean) and TapeStation (Agilent) instruments, respectively, discarding two samples. RNA of highest quality was selected for amplification, labeling, and hybridization on Affymetrix HTA 2.0 Genechips using the Affymetrix WT PLUS kit (Affymetrix Inc.) at the European Institute for Systems Biology and Medicine in Lyon. Measures of probes' intensity were then processed at the probe-set level for RMA normalization using Expression Console v1.4. Transcripts were annotated using version 35 of Affymetrix annotation. In addition, automated cell counts were obtained by flow cytometry performed at the Karolinska University Hospital Laboratory in Stockholm, Sweden.

## Statistical Analyses

**Analysis of cytokine serum levels.** The Mann-Whitney *U*-test was used to compare the distribution of cytokines between the group with asthma and control groups. Linear regression models were applied to estimate effects of air pollution exposure on the levels of serum cytokines, the latter being log-transformed to better approximate normal distribution. Adjustment for body mass index of the child at 8 years of age, exposure to secondhand tobacco smoke (SHS) *in utero*, and/or infancy (defined as maternal smoking at least one cigarette per day at any point of time during the pregnancy and/or at the age of 2 mo), current SHS at 8 and 16 y of age (any of the parents smoked daily at the time of respective follow-up), parental education at baseline (low/medium/high), as well as older siblings did not influence the results and were therefore not included. The final models were adjusted for child's sex, doctor's diagnosis of asthma ever up to 8 y of age, and municipality at birth. Stratified analyses by asthma status, child's sex, and SHS exposure *in utero* and/or child's infancy, as well as analyses including interaction terms representing the product of air-pollutant exposure and stratifying variables, were performed to evaluate potential effect modification. In addition, allelic association of the concentration of IL-6 was tested at 17 SNPs within the *IL6* gene typed in 470 children as part of a GWAS study (Melén et al. 2013). The dbSNP "by gene" function was used to identify all *IL6*-associated SNPs (<http://www.ncbi.nlm.nih.gov/variation/view/>).

**Analysis of cytokine transcript levels.** Differential expression of the studied cytokine transcripts in relation with early-life and current air pollution exposure was tested using data obtained as part of the 16-y-old age group follow-up by means of linear regression analysis adjusted for child's sex, DD asthma ever up to 16 y of age, municipality at birth, and measured cell counts.

Furthermore, for IL-6 we evaluated whether the relationship of *IL6* expression with NO<sub>2</sub> exposure differed depending on the genotype status by means of stratified analysis. Analyses of gene expression data were run in "limma" Bioconductor package (Ritchie et al. 2015) in R (version 3.3.1; R Development Core Team).

Air pollution levels were entered as continuous variables without transformation. Estimated air pollution effects were calculated as a change in the log level of an outcome blood parameter for increment of 10 µg/m<sup>3</sup> of NO<sub>2</sub> exposure, and 5 µg/m<sup>3</sup> of PM<sub>10</sub> exposure. Change in the outcome was back-transformed and presented as percentage. Statistical analyses were performed with STATA software (release 13.1; StataCorp).

## Results

A total of 670 children with serum inflammatory markers measurements at 8 y of age were included in the study. The distribution of selected characteristics and air pollution exposure levels in the study population is provided in Table 1. The mean local contribution above regional background to the children's outdoor TRAP exposure levels during the first year of life, and one year prior to follow-up of the 8-y-old group, respectively, was 16.3 and 8.1 µg/m<sup>3</sup> for NO<sub>2</sub>, and 6.0 and 4.3 µg/m<sup>3</sup> for PM<sub>10</sub>. The correlations between estimated NO<sub>2</sub> and PM<sub>10</sub> concentrations in the various time periods were high ( $r = 0.97$  and  $r = 0.96$  between NO<sub>2</sub> and PM<sub>10</sub> exposure levels during the first year of life and one year before the follow-up at 8 y of age, respectively). Given the selection frame, we had more children with asthma in the current study sample in comparison with the entire cohort.

Table 2 provides a summary of serum cytokine concentrations in children with and without doctor's diagnosis of asthma ever up to 8 y old. Levels of IL-10 and IL-8 differed between children with and without an asthma diagnosis (Mann-Whitney *U*-test,

**Table 1.** Distribution of selected characteristics for the BAMSE children included in the analysis ( $n = 670$ ).

Characteristic	N (%)
Males	367 (54.8)
Doctor's diagnosis of asthma ever up to 8 y	242 (36.1)
Municipality at birth:	
Stockholm	225 (33.7)
Järfälla	174 (26.1)
Solna	190 (28.4)
Sundbyberg	79 (11.8)
Age at follow-up, years, mean ± SD	8.3 ± 0.5
NO <sub>2</sub> during infancy (µg/m <sup>3</sup> ), mean/median (min-max)	16.3/15.8 (0.7–40.1)
PM <sub>10</sub> during infancy (µg/m <sup>3</sup> ), mean/median (min-max)	6.0/5.8 (0.3–16.5)
NO <sub>2</sub> at 8 years (µg/m <sup>3</sup> ), mean/median (min-max)	8.1/6.2 (0.4–29.9)
PM <sub>10</sub> at 8 years (µg/m <sup>3</sup> ), mean/median (min-max)	4.3/3.5 (0.2–14.3)
Maternal smoking during pregnancy and/or infancy	77 (11.5)
Asthma treatment <sup>a</sup> during the last 12 mo prior to 8-y follow-up	155 (23.2)

<sup>a</sup>Asthma treatment = any treatment for breathing difficulties in the last 12 months.

$p < 0.05$ ), which may be attributed to the difference in the distribution of cytokine concentrations between these groups, whereas for IL-6 concentration the difference was less clear ( $p = 0.09$ ) (see Figure S1). Pairwise correlation of cytokine levels was moderate to null (Pearson correlation coefficient ranging from  $r = 0.48$  for IFN $\gamma$  and IL-10 to  $r = -0.001$  for IL-1 $\beta$  and IL-12p70, data not shown).

A 10 µg/m<sup>3</sup> increase of exposure to NO<sub>2</sub> during infancy was significantly associated with an increase of 13.6% (95% CI: 0.8, 28.1%) in IL-6 concentration (Table 3). Positive but not statistically significant associations were also observed with IFN $\gamma$  and IL-10, while the rest of the tested cytokines tended to be negatively associated with early life NO<sub>2</sub> exposure. Association of current NO<sub>2</sub> exposure at 8 y with IL-6 levels was also positive, but no longer significant. No clear association of current exposure with other considered cytokines was detected. Similar trends were observed for PM<sub>10</sub> exposure (Table S1).

In stratified analysis by asthma status, early-life NO<sub>2</sub> exposure was significantly associated with increase in IL-6 and IL-10 levels among children with an asthma diagnosis as follows: 28.8% (95% CI: 1.2, 63.8%) and 27.8% (4.6; 56.2%), respectively, although no significant interaction between air pollution exposure during infancy and asthma status was observed (Table 4). However, an inverse relationship between concurrent NO<sub>2</sub> exposure at 8 y of age and concentrations of IL-4 and TNF $\alpha$  was observed in children with asthma ( $p$ -value for interaction with DD asthma = 0.03 and 0.003, respectively). Similar results were seen for PM<sub>10</sub> (Table S2).

Further stratified analysis suggested stronger association of NO<sub>2</sub> exposure during infancy with IL-6 concentrations in boys: 20.8% increase (95% CI: 1.2, 44.2%), in comparison with girls: 6.6% (−9.4; 25.3%), as well as negative association with IL-2 in girls: 24.5% decrease (−41.6; −2.4%), but not in boys: 20.0% increase (−10.3; 60.6%) per 10 µg/m<sup>3</sup> increase of NO<sub>2</sub> exposure (Table S3). However,  $p$ -values for interaction between NO<sub>2</sub> and sex were not significant ( $p > 0.05$ ). NO<sub>2</sub> exposure at 8 y of age was significantly associated with increased IL-10 levels in children exposed to maternal smoking *in utero* and/or during infancy: 38.4% (95% CI: 9.7, 74.5%) per 10 µg/m<sup>3</sup> increase of exposure to NO<sub>2</sub> ( $p$ -value for interaction = 0.04). Similar patterns were observed with PM<sub>10</sub> exposure (Table S4).

In sensitivity analyses, excluding children currently taking asthma medication had little effect on the estimated effects on

**Table 2.** Distribution of serum inflammatory cytokines concentration stratified by doctor's diagnosis of asthma ever up to 8 y.

Cytokines (pg/ml)	DD asthma ever up to 8 y (N = 242)					No DD asthma ever up to 8 y (N = 416)					p-value <sup>a</sup>
	Mean	Median	Min	Max	IQR	Mean	Median	Min	Max	IQR	
IFN $\gamma$	7.47	3.97	1.31	100.3	3.12	6.28	4.02	1.65	124.0	2.32	0.54
IL-1 $\beta$	0.05	0	0	1.85	0	0.09	0	0	8.11	0	0.67
IL-10	0.29	0.23	0.08	2.12	0.13	0.30	0.21	0.05	7.98	0.12	0.03
IL-12p70	0.13	0.11	0	1.48	0.10	0.14	0.11	0	1.59	0.10	0.55
IL-13	0.48	0.47	0	1.98	0.39	0.53	0.50	0	3.17	0.41	0.41
IL-2	0.24	0.15	0	8.71	0.13	0.22	0.15	0	14.09	0.13	0.58
IL-4	0.04	0.03	0	0.87	0.04	0.04	0.03	0	0.49	0.04	0.59
IL-6	0.34	0.27	0.04	2.06	0.21	0.29	0.24	0.06	1.49	0.17	0.09
IL-8	2.03	1.62	0.46	28.81	0.90	2.21	1.77	0.60	27.59	1.13	0.003
TNF $\alpha$	1.18	1.15	0.50	4.72	0.45	1.17	1.11	0.43	3.56	0.48	0.66

Note: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

<sup>a</sup>p-values for differences between children with and without asthma using the Mann-Whitney U-Test.

IL-6 levels (Table S5). The association between early-life traffic-related NO<sub>2</sub> exposure and IL-6 levels was comparable after NO<sub>2</sub> exposure at 8 y of age has been added to the same model, although the estimate was now of borderline statistical significance (Table S6). The correlation between early and current NO<sub>2</sub> exposure levels was moderate ( $r=0.41$ ). Similar results were observed for PM<sub>10</sub> exposure (data not shown).

No allelic association was observed between SNPs within the *IL6* gene and levels of IL-6 in blood, nor was evidence of interaction between these SNPs and air pollution exposure on cytokine levels observed (data not shown). Using a sample of 16-y-olds with available transcriptomic data, we examined whether air pollution exposure influenced the expression levels of genes encoding the studied cytokines. Early-life NO<sub>2</sub> exposure was significantly associated with increased expression of *IL10*, *IL13*, and *TNF* but not *IL6* at 16 y of age (Table 5), whereas the association with PM<sub>10</sub> exposure during infancy appeared to be significant only for *IL10* (Table S7). Current NO<sub>2</sub> and PM<sub>10</sub> exposures at 16 y of age were linked to differential expression of *IL1 $\beta$* , *IL12A*, as well as *IL13*. Additional analysis of gene-environment interaction highlighted significant interacting SNP  $\times$  NO<sub>2</sub> effects on *IL6* expression for one *IL6* SNP, rs2069837 (interaction p-value = 0.03) (Table S8). A stratified analysis showed that the effect of NO<sub>2</sub> exposure at 16 y of age on the *IL6* expression was significant only in subjects with the *GG* genotype of rs2069837 ( $p=0.007$ ) but not in subjects with the *AG* ( $p=0.13$ ) genotype.

## Discussion

Exposure to TRAP during the first year of life was in this study related to an increased serum level of marker of inflammation IL-6 in children 8 y of age. This association tended to be

stronger in boys, as well as in individuals with doctor's diagnosis of asthma. We also found association of air pollution exposure with elevated levels of IL-10 in children with diagnosis of asthma, as well as in children exposed to maternal smoking during pregnancy and/or infancy. Finally, air pollution exposure during infancy also correlated with *IL10*, *IL13*, and *TNF* transcript levels measured at 16 y of age.

Local inflammation together with oxidative stress has been suggested as a potential biological pathway explaining higher susceptibility of individuals with asthma to air pollution exposure (Auerbach and Hernandez 2012). Growing evidence also indicates that systemic inflammation plays an important role in the pathogenesis of asthma (Wouters et al. 2009). We investigated a wide panel of inflammatory biomarkers to evaluate the effects of long-term TRAP exposure on systemic inflammatory status in children. IL-6 has been associated with diseases characterized by systemic reactions (Kishimoto 2006) and mediates inflammation, particularly in the pathogenesis of pulmonary diseases (Pedroza et al. 2011; Rincon and Irvin 2012). Our results are consistent with other studies on adults showing elevated levels of IL-6 in relation to short- (Tsai et al. 2012) and long-term exposure to NO<sub>2</sub> (Chuang et al. 2011; Panasevich et al. 2009), as well as PM<sub>2.5</sub> (Hajat et al. 2015; Hoffmann et al. 2009). However, only a few epidemiological studies have evaluated influence of exposure to air pollution on markers of systemic inflammation in children. In the German GINI/LISA cohorts, current exposure to NO<sub>2</sub> was associated with higher pro-inflammatory cytokine responsiveness (adjusted means ratio 2.22, 95% CI: 1.22–4.04, for increase in IL-6 per 2.7  $\mu\text{g}/\text{m}^3$  NO<sub>2</sub>) in whole blood of 6-y-old children with asthma, with significant interaction between asthma status and NO<sub>2</sub> exposure (Klümper et al. 2015). Similarly, in a pilot study from Mexico, 6-y-old children with a lifetime residency in the highly polluted Mexico City had significantly higher concentrations of IL-6 in comparison with a control group from the municipality of Polotitlán in Mexico State, Mexico, with levels below the current U.S. National Ambient Air Quality Standards (Calderon-Garcidueñas et al. 2013). Both studies had relatively small sample sizes and did not examine the effects of early-life exposure. In contrast, other studies conducted in children reported a lack of association with inflammatory markers (Armijos et al. 2015; Brown et al. 2012). These negative results might partly be explained by the use of proximity to the nearest road as a proxy for individual air pollution exposure, which may result in more exposure misclassification than in studies with more detailed exposure assessment.

Experimental studies showed significantly higher time- and dose-dependent expression of *IL6* in cultured human lung epithelial cells following fine particulate exposure (Bach et al. 2015; Liu et al. 2014; Shang et al. 2013; Totlandsdal et al. 2010). Our finding of elevated serum IL-6 levels could not be supported with

**Table 3.** Associations between NO<sub>2</sub> exposure and blood cytokine levels in the BAMSE children.

Cytokine	NO <sub>2</sub> during infancy		NO <sub>2</sub> at 8 y	
	N	Estimate <sup>a</sup> (95% CI)	N	Estimate <sup>a</sup> (95% CI)
IFN $\gamma$	655	11.1 (–3.3; 27.7)	644	1.3 (–8.4; 11.9)
IL-1 $\beta$	145	–5.3 (–55.8; 102.8)	142	1.3 (–44.3; 84.2)
IL-10	655	5.6 (–6.0; 18.6)	644	4.1 (–4.2; 13.1)
IL-12p70	621	–8.4 (–24.3; 10.9)	610	1.8 (–11.3; 16.9)
IL-13	585	–1.5 (–15.5; 14.8)	576	1.7 (–9.1; 13.7)
IL-2	627	–5.5 (–22.3; 14.9)	616	0.8 (–12.4; 15.9)
IL-4	577	–5.8 (–24.8; 18.1)	566	–13.2 (–25.8; 1.6)
IL-6	655	13.6 (0.8; 28.1)	644	4.7 (–3.9; 14.2)
IL-8	655	0.1 (–9.7; 10.9)	644	–5.8 (–12.5; 1.4)
TNF $\alpha$	655	–0.8 (–7.0; 5.9)	644	–0.1 (–4.6; 4.7)

Note: NO<sub>2</sub>, nitrogen dioxide; IL, interleukin; IFN, interferon.

<sup>a</sup>Results are obtained by means of linear regression analyses and presented as percentage change in serum concentration of cytokines per 10  $\mu\text{g}/\text{m}^3$  increase of NO<sub>2</sub> exposure. Adjusted for child's sex, DD asthma ever up to 8 y of age, and municipality at birth.

**Table 4.** Associations between NO<sub>2</sub> exposure and blood cytokine levels stratified by doctor's diagnosis of asthma ever up to 8 y of age in the BAMSE children.

Exposure	Cytokine	Asthmatics		Nonasthmatics		<i>p</i> -value <sup>b</sup> for interaction
		<i>N</i>	Estimate <sup>a</sup> (95% CI)	<i>N</i>	Estimate <sup>a</sup> (95% CI)	
NO <sub>2</sub> during infancy	IFN $\gamma$	242	27.5 (-3.7; 68.9)	413	5.3 (-10.0; 23.0)	0.33
	IL-1 $\beta$	51	-1.3 (-68.1; 206.0)	94	-11.5 (-68.2; 145.9)	0.57
	IL-10	242	27.8 (4.6; 56.2)	413	-2.2 (-15.2; 12.8)	0.61
	IL-12p70	233	-2.1 (-30.9; 38.8)	388	-10.0 (-28.6; -28.6)	0.40
	IL-13	215	0.3 (-20.6; 26.8)	370	-2.7 (-20.3; 18.8)	0.75
	IL-2	234	-22.7 (-47.9; 26.8)	393	2.6 (-17.7; 27.9)	0.57
	IL-4	217	-29.7 (-54.6; 9.0)	360	6.7 (-17.8; 38.7)	0.12
	IL-6	242	28.8 (1.2; 63.8)	413	8.6 (-5.2; 24.3)	0.73
	IL-8	242	-4.1 (-21.2; 16.8)	413	1.8 (-9.9; 14.3)	0.47
	TNF $\alpha$	242	0.7 (-10.8; 13.7)	413	-0.9 (-8.3; 7.0)	0.25
NO <sub>2</sub> at 8 y	IFN $\gamma$	234	-1.7 (-21.1; 22.6)	410	2.1 (-8.9; 14.4)	0.16
	IL-1 $\beta$	48	114.5 (-18.1; 461.7)	94	-34.8 (-70.6; 44.8)	0.13
	IL-10	234	1.6 (-13.0; 18.7)	410	6.0 (-4.4; 17.6)	0.13
	IL-12p70	225	-8.3 (-29.7; 19.6)	385	8.4 (-8.6; 28.5)	0.17
	IL-13	208	6.2 (-11.5; 27.5)	368	0.5 (-13.4; 16.5)	0.68
	IL-2	226	-11.6 (-34.8; 19.8)	390	5.2 (-10.4; 23.5)	0.29
	IL-4	209	-34.4 (-52.5; -9.3)	357	-1.4 (-18.2; 18.8)	0.03
	IL-6	234	-1.7 (-18.6; 18.7)	410	8.6 (-1.6; 19.8)	0.19
	IL-8	234	-12.6 (-24.7; 1.3)	410	-4.0 (-12.1; 5.0)	0.26
	TNF $\alpha$	234	-7.7 (-15.9; 1.3)	410	3.9 (-1.8; 9.8)	0.003

Note: NO<sub>2</sub>, nitrogen dioxide; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

<sup>a</sup>Results are obtained by means of linear regression analyses and presented as percentage change in serum concentration of cytokines per 10  $\mu\text{g}/\text{m}^3$  increase of NO<sub>2</sub> exposure in subjects with asthma and children without asthma, respectively). Adjusted for child's sex, and municipality at birth.

<sup>b</sup>*p*-value for interaction term representing the product of NO<sub>2</sub> exposure and DD of asthma.

available functional data on gene expression. The fact that serum cytokines were measured in a sample of 8-y-olds and transcriptomic analysis was performed in 16-y-olds might have contributed to the different results. We could, however, see a significant interacting effect of *IL6* SNP, rs2069837, with current NO<sub>2</sub> exposure at 16 y of age on *IL6* expression. However, given the relatively small sample size of individuals with both genetic and mRNA data, our results may be underpowered and should, therefore, be interpreted with caution. Interestingly, this SNP has been suggested as a marker for a susceptibility to inflammation-mediated cancers due to a mechanism altering the *IL6* mRNA expression (Shi et al. 2013; Zhou et al. 2016). Thus, Zhou and colleagues found that the increase of *IL6* mRNA expression was associated with the increasing number of the *G* allele, which is similar to our findings. It is, however, important to note that these analyses were mainly based on Chinese populations, and it remains to be investigated whether the findings can be extrapolated to other ethnic groups.

IL-10, a pleiotropic cytokine released from various types of cells, including mononuclear cells, macrophages, T-lymphocytes, B-lymphocytes, and mast cells, is one of the most important anti-inflammatory cytokines regulating the progression of the immune response by suppressing the production of a number of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 (Chung 2001; Dobrev et al. 2009). Studies have shown that IL-10 is constitutively produced in the lungs and peripheral blood of healthy subjects, which may help prevent the development of inflammation in the healthy state, whereas subjects with asthma are characterized by decreased concentrations of IL-10 (Chung 2001). This finding is in line with our results demonstrating higher levels of serum IL-10 in children without asthma in comparison with children who have had an asthma diagnosis. The increased level of IL-10 after exposure to air pollutants such as diesel exhaust in the airways of patients with asthma indicates that air pollution may induce subtle changes in airway immunobiology (Leem et al. 2005). Furthermore, several studies reported

**Table 5.** Associations between NO<sub>2</sub> exposure and gene expression in 16-y-old BAMSE children (*N* = 238).

Probe ID	Gene	NO <sub>2</sub> during infancy			NO <sub>2</sub> at 16 y		
		LogFC <sup>a</sup>	<i>p</i> -value	FDR <sup>b</sup> <i>p</i> -value	LogFC <sup>a</sup>	<i>p</i> -value	FDR <sup>b</sup> <i>p</i> -value
TC12001696.hg.1	<i>IFNG</i>	0.04	0.41	0.49	0.06	0.29	0.69
TC02002219.hg.1	<i>IL1B</i>	-0.06	0.30	0.42	-0.17	0.01	0.09
TC01003766.hg.1	<i>IL10</i>	0.09	0.003	0.06	0.05	0.13	0.59
TC03000870.hg.1	<i>IL12A</i>	0.04	0.13	0.19	0.08	0.02	0.09
TC05001997.hg.1	<i>IL12B</i>	0.03	0.33	0.43	0.02	0.66	0.79
TC05000640.hg.1	<i>IL13</i>	0.07	0.01	0.07	0.08	0.02	0.09
TC04001520.hg.1	<i>IL2</i>	0.05	0.13	0.19	0.004	0.93	0.93
TC05000641.hg.1	<i>IL4</i>	0.03	0.44	0.49	-0.01	0.86	0.93
TC07000137.hg.1	<i>IL6</i>	0.001	0.97	0.97	0.02	0.54	0.69
TC04000408.hg.1	<i>IL8</i>	-0.01	0.84	0.89	0.01	0.89	0.93
TC6_apd_hap1000036.hg.1	<i>TNF</i>	0.09	0.04	0.07	0.06	0.28	0.69
TC06000371.hg.1 <sup>c</sup>	<i>TNF</i>	0.08	0.04	0.07	0.03	0.51	0.69

Note: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

<sup>a</sup>Results are obtained by means of linear regression analyses and presented per 10  $\mu\text{g}/\text{m}^3$  increase of NO<sub>2</sub> exposure. Adjusted for child's sex, DD asthma ever up to 16 y of age, municipality at birth, and measured cell counts. LogFC = logarithm fold-change (one unit of the logFCs translates to a two-fold change in expression).

<sup>b</sup>FDR = false discovery rate correction based on 18 probesets.

<sup>c</sup>Seven alternatives haplotypes assemblies of this TNF probeset are available (apd, cox, dbb, mann, mcf, qbl and ssto) and bear similar log<sub>2</sub> intensity values.

increased *IL10* expression and IL-10 plasma levels in children living in highly polluted areas (Calderon-Garcidueñas et al. 2009; Dobrev et al. 2015), which is also supported by *in vitro* studies (Saito et al. 2002).

IL-10 may also play a role in tobacco smoke-induced inflammation. A study of adults showed that IL-10 levels were significantly correlated with urinary 1-OHPG and cotinine, constituting biomarkers of polycyclic aromatic hydrocarbon and SHS exposure, respectively, suggesting that these exposures alter immunological responses leading to increased IL-10 (Leem et al. 2005), which supports our observation of a stronger association between TRAP exposure and IL-10 concentrations in children exposed to tobacco smoke *in utero* and/or early in life. Interestingly, studies considering both tobacco smoke exposure and air pollution exposure demonstrate that tobacco smoke exposure in early life might lead to increased vulnerability of the lungs to the effects of air pollution (Sonnenschein-van der Voort et al. 2012). A synergistic effect of these exposures has also been shown for the molecular changes in the genes involved in the immune function (e.g., significant hypermethylation and decreased expression of *IFN $\gamma$*  in effector T-cells and *FOXP3* in regulatory T-cells), in comparison with each exposure alone, suggesting a potential mechanism explaining how these exposures may influence the development and progression of asthma and allergies (Kohli et al. 2012).

The strengths of our study include a relatively large and well-characterized study population, detailed long-term air pollution exposure assessment, precise measurements of serum cytokine concentrations, and evaluation of effect modification. Furthermore, the availability of genetic and transcriptomic data provided a unique opportunity to investigate the functional importance of our findings. In addition, because we aimed to investigate the potential effect modification of long-term TRAP exposure by asthma status, using a study sample enriched with children who had been diagnosed with asthma was also advantageous.

The individual exposure estimates for each child were derived from a time- and space-resolved dispersion model with street canyon addition for addresses in the most polluted street segments, which has been successfully applied in a number of previous studies (Gruzjeva et al. 2012; Schultz et al. 2016). Validation of dispersion models against actual measurements suggests good model performance in predicting ambient concentrations of  $\text{NO}_2$  ( $r^2 = 0.71$ ),  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  ( $r^2 = 0.61$ ) (Eneroth et al. 2006), as well as  $\text{NO}_x$  ( $r^2 = 0.68$ ) (Korek et al. 2016). Furthermore, to increase the precision in the exposure assessment, we accounted for time-activity patterns, considering the time children spent at home, daycare, and school. We were able to investigate several duration aspects of long-term exposure, such as exposure during the first year of life and current exposure during 12 months prior to blood sampling. The strongest effect was observed in relation to exposure during infancy, which is in line with previous studies indicating that the early-life period represents a critical time window for the development and maturation of several biological systems, including the lung and immune system, and that air pollutants may impair adaptive immune responses, thus contributing to negative health effects throughout childhood (Clark et al. 2010; Gehring et al. 2015; Gruzjeva et al. 2012; Schultz et al. 2016). Additionally, the exposure to higher concentrations of combustion-related air pollutants during early childhood than exposure later in life in our study might also have contributed to the higher risks associated with exposure during this period. An alternative explanation might be that the exposure classification method based on home address is more accurate early in life, before children start to go to child care and school. In addition, some misclassification of exposure may have occurred

because estimated outdoor exposure levels may not be equivalent to true personal exposure. However, because air pollution exposure was assessed independently from health outcomes and potential confounders, possible misclassification of exposure is likely to be nondifferential, which would tend to attenuate the associations.

Our study also has limitations. One limitation is the lack of longitudinal data on markers of inflammation and gene expression, and such data were available only for air pollution exposure. Another limitation is limited power in some analyses, mainly related to subgroups and interactions. In addition, given the multiple comparisons made, statistically significant results might be due to chance alone. On the other hand, because the significance tests were used to evaluate some *a priori* hypotheses based on existing literature, such as whether air pollution exposure influences IL-6 levels in serum, individual test results were not adjusted for multiple comparisons (Perneger 1998). Another limitation is the difficulty to attribute the observed associations to a specific air pollution component such as ( $\text{NO}_2$  or  $\text{PM}_{10}$ ) because of the strong correlation between the levels of these traffic-related pollutants. Finally, we investigated a limited number of inflammatory markers in relation to air pollution exposure without considering patterns or complex interactions between the markers. Future large-scale studies should attempt to explore a broader range of inflammatory cytokines in repeated samples to expand our understanding of underlying mechanisms and interactions between cytokines in the inflammatory response following air pollution exposure.

This study adds to the growing body of evidence demonstrating pro-inflammatory effects of exposure to TRAP. This finding is important from a public-health perspective, given that even slight increases in the levels of inflammatory cytokines may represent a health threat for the population by increasing risks for respiratory and other morbid conditions. Furthermore, the observed association of elevated inflammatory cytokines with long-term exposure to TRAP in an area with comparatively clean air such as Stockholm supports the importance of further lowering urban air pollution levels.

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