



Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children[☆]



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ABSTRACT

Epidemiological studies indicate that asthmatic children are more susceptible to traffic-related air pollution exposure than non-asthmatic children. Local and systemic inflammation in combination with oxidative stress have been suggested as a possible susceptibility factor.

We investigated effect modification by asthma status for the association between air pollution exposure and systemic effects using whole blood cytokine responsiveness as an inflammatory marker.

The study was nested within the two German birth cohort studies GINIplus and LISApplus and initially designed as a random sub-sample enriched with asthmatic children. Using data from 27 asthmatic and 59 non-asthmatic six-year-old children we measured the production of Interleukin-6 (IL-6), IL-8, IL-10, monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) in whole blood after ex-vivo stimulation with urban particulate matter (EHC-93). Air pollution exposure (nitrogen dioxide (NO₂), nitrogen oxides (NO_x), particulate matter with an aerodynamic diameter < 10 µm (PM₁₀), particulate matter with an aerodynamic diameter < 2.5 µm (PM_{2.5mass}), coarse particulate matter (PM_{coarse}) and PM_{2.5absorbance} (PM_{2.5abs})) was modelled for children's home addresses applying land-use regression. To assess effect modification by asthma status linear regression models with multiplicative interaction terms were used.

In asthmatics exposure to NO₂ was associated with higher production of pro-inflammatory cytokines: adjusted means ratio (MR) 2.22 (95% confidence interval 1.22–4.04) for IL-6 per 2.68 µg/m³ NO₂. The interaction term between asthma status and NO₂ exposure was significant. Results for NO_x, PM₁₀, PM_{2.5mass} and PM_{2.5abs} were in the same direction. No association between air pollution and cytokine responsiveness was found in the group of non-asthmatic children and in the overall group.

Traffic-related air pollution exposure is associated with higher pro-inflammatory cytokine responsiveness in whole blood of asthmatic children.

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1. Introduction

Asthma is one of the most common chronic diseases in childhood (Sennhauser et al., 2005). Many studies have investigated the role of traffic-related air pollution (TRAP) in childhood asthma and

there is sufficient evidence for a causal association between TRAP and the exacerbation of asthma symptoms in children (Health Effects Institute, 2010). Whereas there is more evidence from studies investigating short-term effects of air pollution exposure on acute asthma exacerbation (Mann et al., 2010; Segala et al., 1998), the effects of long-term exposure to air pollution in asthmatics are less well investigated. A few studies indicate that long-term air pollution exposure is associated with an increase in chronic asthma symptoms (Braun-Fahrlander et al., 1997; Heinrich et al., 2000; McConnell et al., 1999 and 2003; Brauer et al., 2007). However, the underlying biological pathways behind these observations have still to be elucidated. In accordance with the

[☆]Take home message: Air pollution exposure is associated with pro-inflammatory cytokine responsiveness in whole blood of asthmatic children.

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oxidative stress hypothesis it has been shown that inhaled particulate matter (PM) induces pro-inflammatory responses in the respiratory system (Behndig et al., 2011; Stenfors et al., 2004; Wessels et al., 2010). Besides local inflammation systemic inflammation plays an important role in asthma pathogenesis (Baines et al., 2011; Wouters et al., 2009). Thus, components of TRAP might trigger the asthmatic immune system via systemic effects. There is strong evidence that systemic effects can be induced by inhaled PM, especially by ultrafine particles (UFPs) and nanoparticles (NPs) (Nemmar et al., 2013). One suggested mechanism is particle translocation from the lungs into the blood circulation (Nakane, 2012) where translocated particles might cause direct systemic effects like the release of pro-inflammatory mediators. The whole blood assay (WBA) is a suitable method to detect an altered stimulus-dependent production of systemic inflammatory markers (inflammatory responsiveness) caused by exposure to various environmental factors (Herberth et al., 2008; Schins et al., 1996). However, no study has previously investigated the association between long-term traffic-related air pollution exposure and whole blood cytokine responsiveness as a marker for systemic effects. We hypothesized that long-term TRAP exposure is associated with a higher pro-inflammatory cytokine response in asthmatic children compared to non-asthmatic children, explaining the higher susceptibility of asthmatics to TRAP. To proof our hypothesis we used a unique approach choosing whole blood cytokine responsiveness after stimulation with urban particulate matter as a marker for inflammatory patterns. Conducting a study on 27 asthmatic and 59 non-asthmatic six-year-old children participating in the population-based German birth cohort studies (GINIplus and LISAplus) we evaluated effect modification by asthma status for the association of long-term residential TRAP exposure with whole blood cytokine responsiveness.

2. Material and Methods

2.1. Study collective

The present study was nested within the regular six year follow-up examination of the two ongoing birth cohort studies GINIplus and LISAplus and included children from the study centre in Wesel. Designs of both studies have been described elsewhere in detail (Zirngibl et al., 2002; Zutavern et al., 2006). As we wanted to investigate effect modification by asthma status our nested study was initially designed as a sub-sample enriched with asthmatic children. Between September 2002 and March 2005 all children with parent-reported physician-diagnosed asthma attending the study centre for the follow-up examination were invited to participate. Parent-reported physician-diagnosed asthma had to be confirmed a priori by a pediatric pulmonologist. Criteria for the diagnosis of asthma were: medical history, physical examination or a long-term asthma medication with a combined use of anti-inflammatory medication and bronchodilator medication and if available data on lung function testing (available for more than 90% of the children). To each confirmed asthma case two control subjects were matched according to sex and the time of the examination. The study was approved by the local Ethics Committee and written consent from parents was obtained.

2.2. Exposure assessment

Air pollution exposure data for nitrogen dioxide (NO_2), nitrogen oxides (NO_x), particulate matter with an aerodynamic diameter $< 10 \mu\text{m}$ (PM_{10}), particulate matter with an aerodynamic diameter $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5\text{mass}}$), coarse particulate matter ($\text{PM}_{\text{coarse}}$) and $\text{PM}_{2.5\text{absorbance}}$ ($\text{PM}_{2.5\text{abs}}$) were collected and modeled for children's

home addresses using land use regression (LUR) models developed in the frame work of the EU project ESCAPE (Cyrys et al., 2012; Eeftens et al., 2012). Briefly, between October 2008 and November 2009 three two-week air pollution measurement campaigns were performed at 40 NO_x measurement sites and 20 PM measurement sites in the study area. Site-specific annual averages were calculated using the average of these three measurement periods and were adjusted for temporal variation using data from a centrally located background reference site which operated continuously throughout the measurement year. Area-specific LUR models were used to estimate exposures and modeled annual averages were then assigned to children's home addresses at the age of six. Validity of the modeled exposure estimates was high: explained variance (R^2) and R^2 cross validation was 88% and 79% for $\text{PM}_{2.5\text{mass}}$, 69% and 63% for PM_{10} , 97% and 95% for $\text{PM}_{2.5\text{abs}}$, 66% and 57% for $\text{PM}_{\text{coarse}}$, 89% and 84% for NO_2 and 88% and 81% for NO_x .

2.3. Whole blood assay (WBA)

The WBA was performed as described previously (Schins et al., 1996), with some minor modifications (see online supplementary file). Ex-vivo stimulation of blood samples with particulate matter (PM tubes) was performed by using EHC-93 urban dust at a final concentration of $100 \mu\text{g/ml}$ in the RMPI-diluted blood. EHC-93 represents a well investigated PM standard that has been collected in Ottawa 1993 from ambient air filter.

2.4. Cytokine measurement

Concentrations of interleukin (IL)-6, IL-8, IL-10, IL-12p70, monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor- α (TNF- α) and interferon-gamma (IFN-gamma) were measured by flow cytometry using the BD CBA Human Soluble Flex Set system (BD Bioscience, Heidelberg, Germany) as described previously (Herberth et al., 2008). Lowest quantification limit for each cytokine was 3 pg/ml. Samples with concentrations below the detection limit were assigned a value that was half of the lowest quantification limit (1.5 pg/ml) and were included in the analysis.

2.5. Statistical analysis

The association between long-term TRAP exposure and blood responsiveness was analyzed by multiple linear regression models (Model 1, see online supplementary file). Air pollution variables were included as continuous variables (increment: interquartile range) in the model. Blood responsiveness was defined as the ratio between cytokine concentration in EHC-93-stimulated and spontaneous (Sp) blood. Using this method we account for inter-individual differences. We stabilized the variance of the residuals with logarithmic transformation of cytokine concentrations (Herberth et al., 2008). To assess effect modification by asthma status in the association between TRAP and cytokine responsiveness we included asthma status (binary) and a multiplicative interaction term of asthma status and air pollution exposure (continuous) in our model (Model 2, see online supplementary file) and calculated the stratum-specific effect estimates for asthmatic and non-asthmatic children by adding the regression coefficients of the exposure term and the respective interaction term. In the main model we adjusted for sex. In the extended model we additionally adjusted for parental school education, siblings, current environmental tobacco smoke at child's home, visible signs of mold or dampness in the home and body mass index of the child. Sensitivity analyses were done including additional variables in our main model: parental allergy, age at blood sampling, season of blood sampling, time between blood sampling and start of

incubation, incubation duration as well as time between the end of incubation and freezing. Furthermore, in sensitivity analyses we excluded children with anti-inflammatory asthma medication and with parental-reported doctor diagnosis of eczema and hay fever at the age of six. Details on the different models and adjustment sets are provided in the online supplementary file. Effect estimates are presented as means ratios with 95% confidence intervals (95%-CI). Means ratios indicate the relative change from the overall geometric mean in blood responsiveness for an interquartile range increase in air pollution exposure. All analyses were completed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characterization of the study population

In total, 86 children had data on blood responsiveness and TRAP exposure and were included in the analysis (Table 1).

13 asthmatic children had detailed data on a combined use of anti-inflammatory medication (mostly inhaled corticosteroids) and bronchodilator medication. 8 asthmatic children did not use any asthma medication. For 6 asthmatics using asthma medication, we did not have detailed information on the kind of medication. Furthermore, 1 non-asthmatic child used bronchodilator medication. Except for prescribed asthma medication and doctor-diagnosed hay fever there were no significant differences between asthmatic and non-asthmatic children (Table 1). However, in the asthmatic group the proportion of children with doctor-diagnosed eczema, with parental allergy, and with high parental education tended to be higher than in the non-asthmatic group.

3.2. Cytokine production in WBA

Ex-vivo production of IL-6, IL-8, TNF-alpha, IL-10 and MCP-1 was increased after stimulation with EHC-93 (Fig. 1). After stimulation there was no elevated production of IL-12p70 and IFN-gamma and more than half of the samples had concentrations below the detection limit. Percentage of EHC-93-stimulated samples with cytokine concentrations below the detection limit (3 pg/ml) was: 5.8% for IL-10, 68.6% for IFN-gamma and 82.6% for IL-12p70. The other cytokines had no stimulated samples with cytokine concentrations below the detection limit.

The blood responsiveness differed markedly between different cytokines (Fig. 1 Ratio PM/Sp Median (IQR)). IL-6 showed the strongest response. After stimulation IL-6 concentrations were on average 287 times higher than before. For IFN-gamma and IL-12p70 blood responsiveness was below one (ratio: 0.5), which indicates that cytokine production after stimulation was lower than spontaneous production. There were no significant differences in blood responsiveness between asthmatic and non-asthmatic children (see online supplementary file Table S1). Excluding 20 children with asthma medication did not change this picture.

3.3. Traffic-related air pollution and whole blood cytokine responsiveness

There was no association between traffic-related air pollution and whole blood cytokine responsiveness in the overall group (Fig. 2). Results did not differ when using different adjustment sets (see online supplementary Table S 2).

3.4. Interaction between traffic-related air pollution and asthma status

With regard to IL-6, IL-8 and TNF-alpha responsiveness the

Table 1

Description of the study population with modelled residential exposure.

	Asthma +	Asthma –
Males, % (N)	66.7 (27)	61.0 (59)
Parental allergy, % (N)	69.2 (27)	47.4 (57)
Siblings, % (N)	63.0 (27)	62.7 (59)
Passive smoking in household in the last 12 month, % (N)	30.0 (27)	39.0 (59)
Asthma medication prescribed at the age of 6, % (N)	48.2*** (27)	1.7 (59)
Doctor-diagnosed eczema at the age of 6, % (N)	15.4* (26)	3.6 (55)
Doctor-diagnosed hay-fever at the age of 6, % (N)	15.4 (26)	1.8 (56)
Dampness or mould in household, % (N)	20.0 (25)	17.2 (58)
Parental school education: < 10 years, % (N)	14.8 (27)	15.5 (58)
= 10 years, % (N)	29.6 (27)	44.8 (58)
> 10 years, % (N)	55.6 (27)	39.7 (58)
Body mass index [kg/m ²] means $\pm$ SD (N)	15.66 $\pm$ 1.69 (27)	15.66 $\pm$ 1.43 (59)
NO ₂ [μ g/m ³] median $\pm$ IQR (N)	22.81 $\pm$ 1.48 (27)	23.18 $\pm$ 3.27 (59)
NO _x [μ g/m ³] median $\pm$ IQR (N)	32.36 $\pm$ 4.72 (27)	33.71 $\pm$ 7.25 (59)
PM _{2.5} [μ g/m ³] median $\pm$ IQR (N)	17.19 $\pm$ 0.67 (27)	17.24 $\pm$ 1.25 (59)
PM ₁₀ [μ g/m ³] median $\pm$ IQR (N)	25.16 $\pm$ 0.90 (27)	25.26 $\pm$ 1.44 (59)
PM _{coarse} [μ g/m ³] median $\pm$ IQR (N)	8.24 $\pm$ 0.48 (27)	8.42 $\pm$ 0.79 (59)
PM _{2.5} abs [10 ^{–5} /m] median $\pm$ IQR (N)	1.13 $\pm$ 0.16 (27)	1.17 $\pm$ 0.21 (59)

Chi-square test comparing children with and without asthma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

stratum-specific means ratios differed between asthmatic and non-asthmatic children (Fig. 3): Means ratios for asthmatic children tended to be greater than 1, whereas means ratios for the group of non-asthmatic children were equal to or slightly below one. In case of IL-6 stratum-specific means ratios for NO₂, NO_x and PM₁₀ were significant for asthmatic children but not for non-asthmatic children. The stratum-specific means ratio for the association between NO₂ and IL-6 responsiveness was 2.22 (95%-CI 1.22–4.04) in asthmatic children versus 0.86 (95%-CI 0.67–1.09) in non-asthmatic children. These data indicate that in asthmatic children whole blood IL-6 responsiveness doubles per 2.68 μ g/m³ (interquartile range) increase in NO₂ exposure. As indicated by asterisks in Fig. 3, graphic for IL-6 the multiplicative interaction term between asthma status and NO₂ and NO_x exposure was also significant (NO₂: means ratio 2.48; 95%-CI 1.32–4.68; $p = 0.005$; NO_x: means ratio 2.06; 95%-CI 1.25–3.39; $p = 0.004$). Similar results were observed for TNF-alpha and to a lesser extent also for IL-8. Results were consistent when using different adjustment sets (online supplementary figure S1 and Table S3).

There was no interaction between air pollution exposure and asthma status with regard to the production of IL-10, IL-12p70, MCP-1 and IFN-gamma (Fig. 3 and Table S3).

Excluding children with anti-inflammatory asthma medication had little impact on the estimated effects of IL-6 and TNF-alpha responsiveness, but as sample size became much smaller (only 73 children without anti-inflammatory asthma medication) confidence limits became wider (online supplementary Table S4 (Model 2sens1)). The same was true for the results after excluding children with parental-reported doctor-diagnosis of eczema or hay-fever at the age of six (online supplementary Table S4 (Model 2sens3)). Including parental allergy in our main model had only little impact on effect estimates (online supplementary Table S4 (Model 2sens2)): estimates were slightly lower but still significant. We also investigated whether differences in age of blood sampling, differences in time between blood sampling and start of incubation and season of blood sampling might change the results.

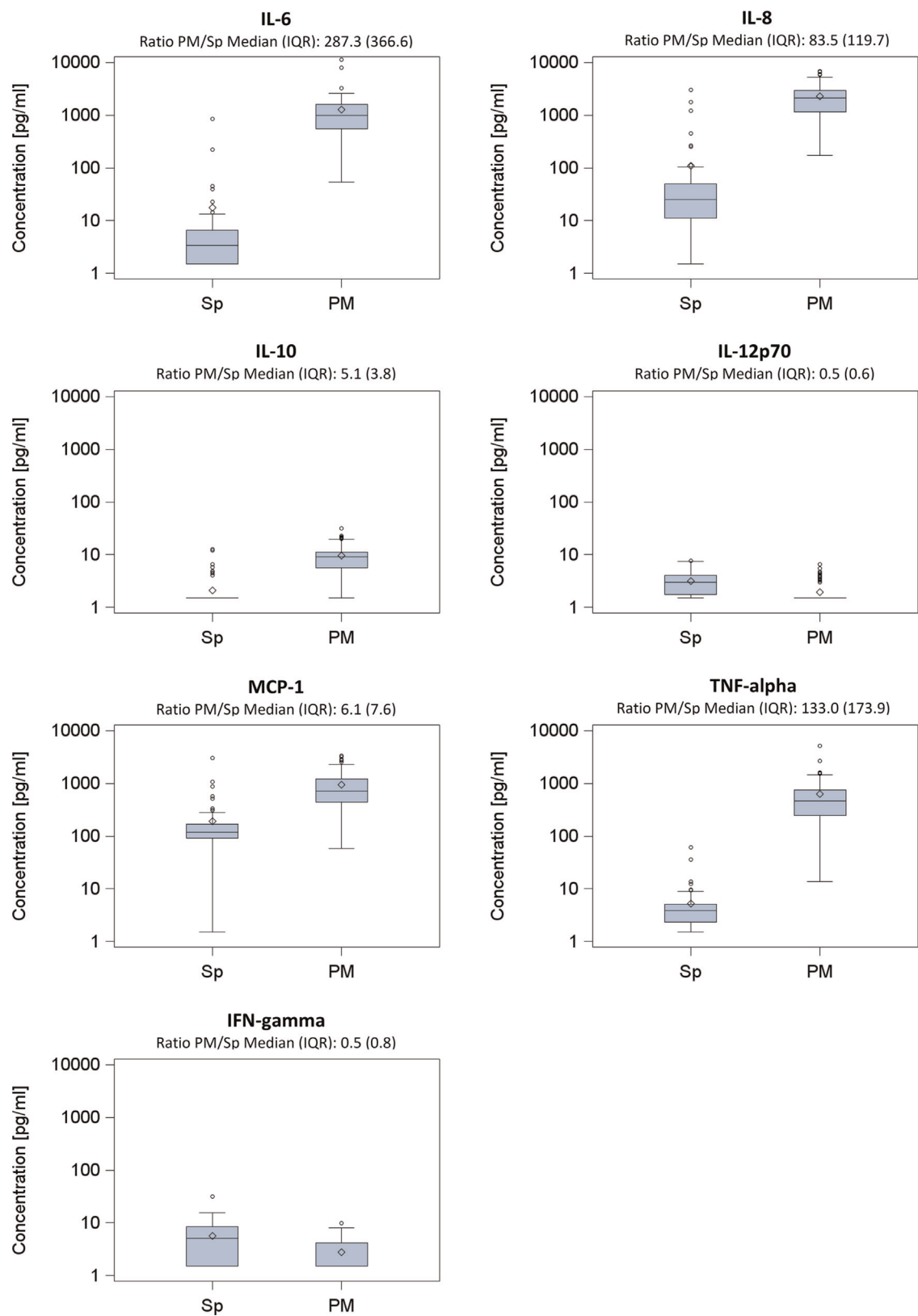


Fig. 1. Cytokine production in the whole blood assay: cytokine concentration in spontaneous (SP) and PM-stimulated (PM) blood samples ($N=86$). Box plots: minimum, 25% percentile, median, 75% percentile and maximum; diamond: arithmetic mean; dots: outliers. Footnote: Ratios between PM-stimulated and spontaneous blood samples, median and interquartile range (IQR).

However, none of them had an effect on the interaction terms of traffic air pollution and asthma status (online supplementary [Table S5](#) (Model 2sens4-6)).

4. Discussion

Our study shows an effect modification by asthma status for the

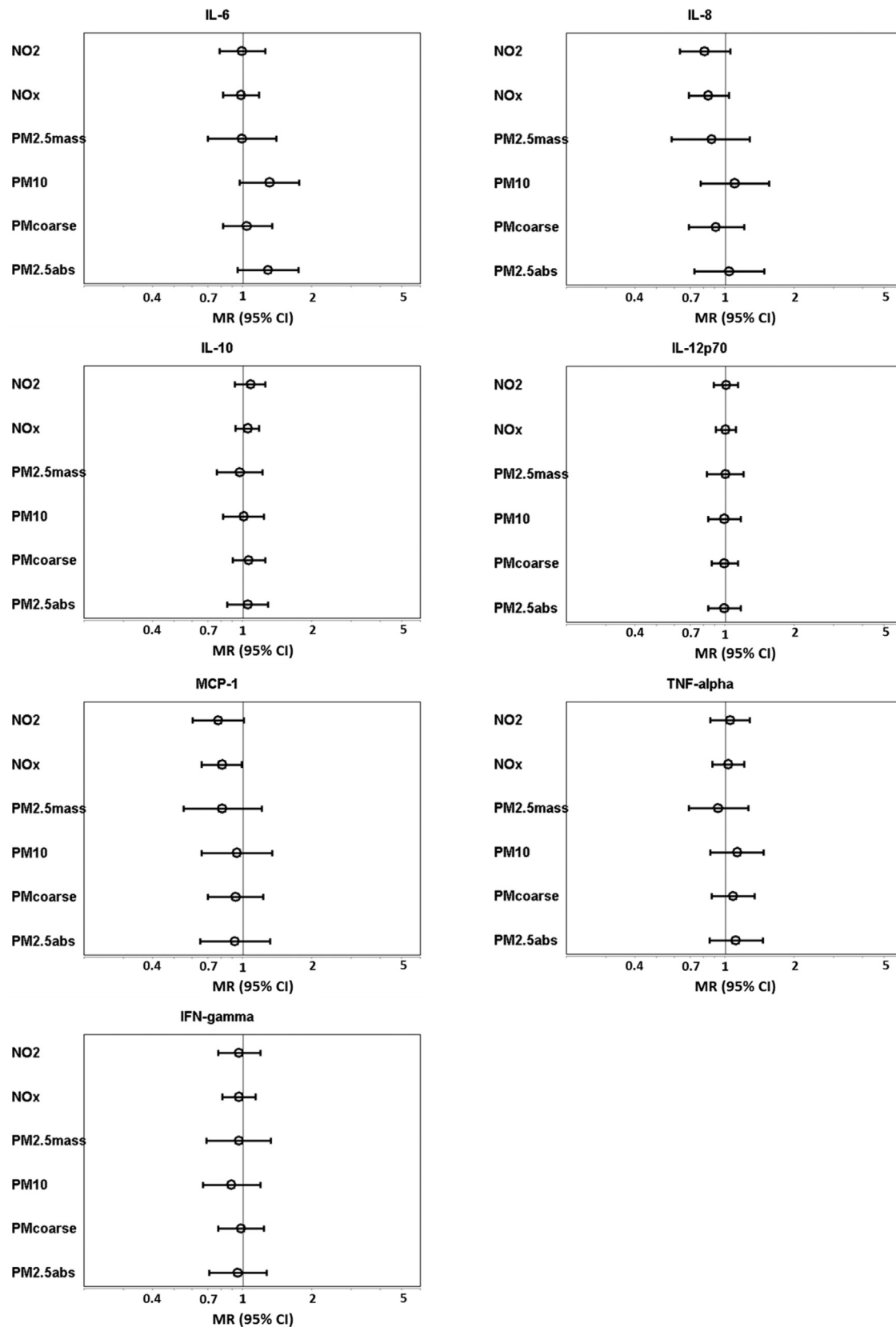


Fig. 2. Effect estimates for the association between traffic-related air pollution and blood responsiveness adjusted for sex and asthma status (N=86)- Results of model 1. Estimates are presented as mean ratios with 95% confidence intervals.

association of long-term residential TRAP exposure with whole blood cytokine responsiveness in six-year-old children. In asthmatic children exposure to air pollution was associated with

higher production of the pro-inflammatory markers IL-6, IL-8 and TNF-alpha. There was no association between air pollution and production of pro-inflammatory markers in non-asthmatic

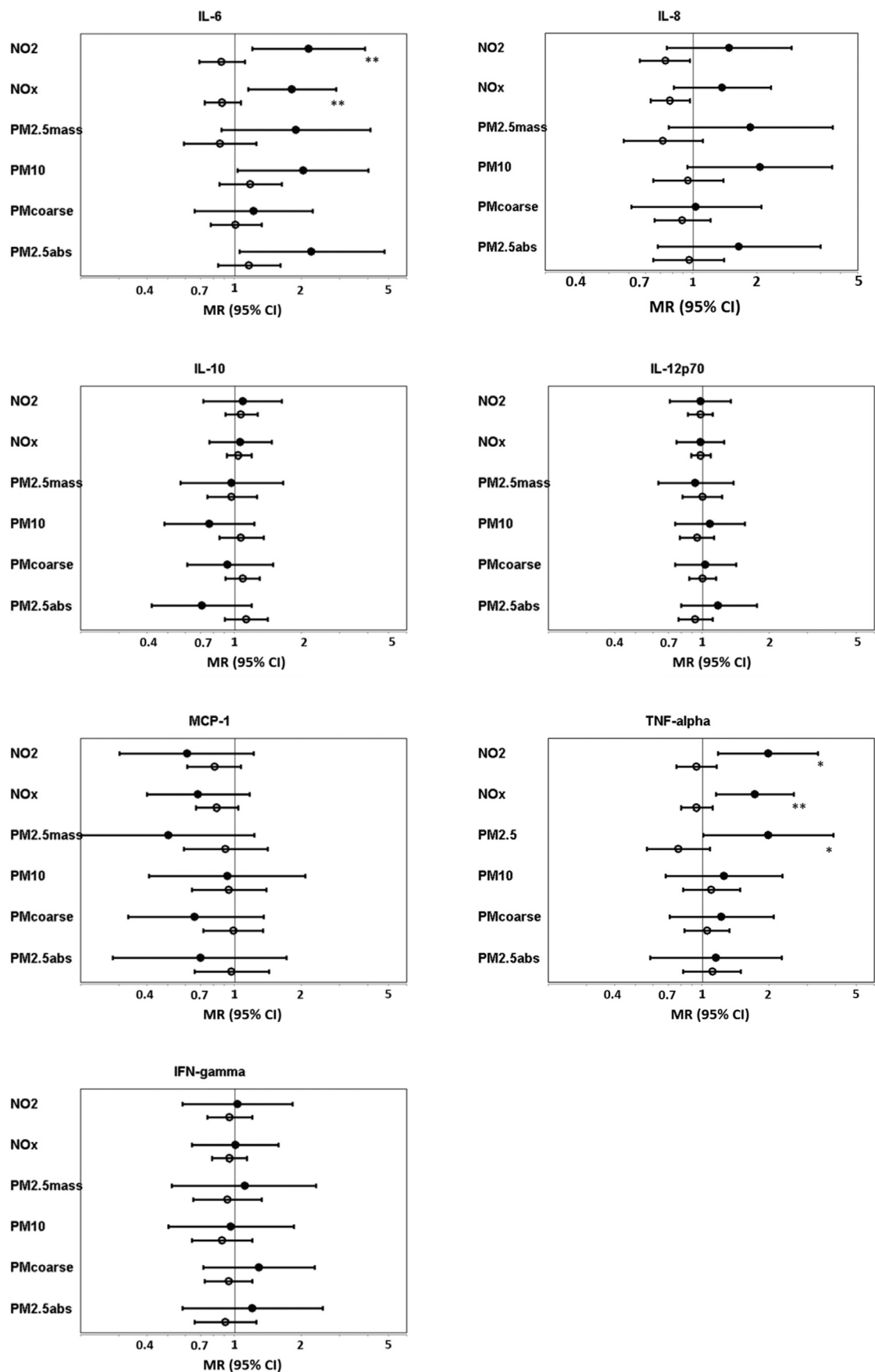


Fig. 3. Stratum-specific effect estimates (calculation based upon model 2 with interaction term) for the association between traffic-related air pollution and blood responsiveness in asthmatic and non-asthmatic children adjusted for sex ($N=86$). Estimates are presented as mean ratios with 95% confidence intervals; black dots: asthmatic children; white dots: non-asthmatic children. P-values for a significant interaction term between asthma status and exposure are indicated by asterisks (* p-value < 0.05; ** p-value < 0.01).

children. No such interactions were found for IL-10, IL-12p70, MCP-1 and IFN- γ . There was no indication of an association between TRAP exposure and cytokine responsiveness in non-asthmatic children.

4.1. Traffic-related air pollution, asthma and inflammation

Although many studies have reported that increases in traffic-related air pollution are associated with asthma outcomes in children (Health Effects Institute, 2010), little is known about the susceptibility of asthmatic children. Whereas effects of short-term air pollution exposure on acute asthma exacerbation have been shown by several studies (Mann et al., 2010; Segala et al., 1998), effects of long-term exposure to air pollution are less well investigated. Only one study so far has indicated an effect of long-term TRAP on asthma hospitalizations in older adults (Andersen et al., 2012). However, some studies indicate an association between long-term TRAP exposure and chronic asthma symptoms in children like an increase in chronic symptoms of bronchitis (Braun-Fahrlander et al., 1997; Heinrich et al., 2000; McConnell et al., 1999 and 2003), an increased risk of wheezing (Brauer et al., 2007) or use of asthma medication (Gauderman et al. 2005). The effect of ambient air pollution on acute asthma exacerbation may initially be a short term effect (Johnston and Sears, 2006). However, it is also likely that long-term exposure to air pollution might act synergistically with other factors (e.g. viral infections) leading to more chronic symptoms (Mc Connell et al., 2003; Künzli et al., 2008; Chauhan and Johnston, 2003).

There is growing evidence that systemic inflammation besides local inflammation plays an important role in asthma pathogenesis (Wouters et al., 2009). We used a unique approach to analyze the systemic effects of long-term traffic-related air pollution exposure by choosing whole blood responsiveness as a marker for altered systemic inflammatory status. Up to know mainly local inflammation in combination with oxidative stress has been suggested as a possible biological pathway explaining higher susceptibility of asthmatic people towards traffic-related air pollution exposure (Auerbach and Hernandez, 2012). Interestingly, several controlled human exposure studies (Stenfors et al., 2004; Behndig et al., 2011) and epidemiological studies (Barraza-Villarreal et al., 2008; Yeatts et al., 2007) could not confirm a TRAP-related enhanced airway inflammation in asthmatic individuals compared to non-asthmatic individuals. Partially, this might be due to a lack of appropriate biomarkers (Balmes, 2011). It is well-known that exposure to diesel exhaust and PM induces a marked systemic inflammatory response (Van Eeden et al., 2001). Furthermore, recent studies have shown that blood inflammatory patterns might help to differentiate between asthma phenotypes and to understand differences in asthma severity (Baines et al., 2011; Nadif et al., 2009; Wouters et al., 2009). Thus, whole blood cytokine production patterns might be an appropriate diagnostic tool helping to explain higher susceptibility of asthmatic children towards long-term TRAP exposure.

IL-6, IL-8 and TNF- α belong to the group of pro-inflammatory cytokines and play an important role in the chronic inflammatory process in asthma (Brightling et al., 2008; Neurath and Finotto, 2011).

There are only very few epidemiological studies investigating the association between traffic-related air pollution and cytokine levels in children (Barraza-Villarreal et al., 2008; Brown et al., 2012). Brown et al. (Brown et al., 2012) observed no association between residential proximity to a major roadway and plasma levels of IL-6, IL-8, IL-10 and TNF- α in asthmatic children. Barraza-Villarreal et al. (Barraza-Villarreal et al., 2008) found a positive association between PM_{2.5} exposure and IL-8 levels in nasal lavage from both asthmatic and non-asthmatic children. In

contrast to our results the effect was stronger for non-asthmatic children than for asthmatic children. However, as cytokine levels in nasal lavage might not be directly comparable with cytokine levels in whole blood interpretation should be done cautiously.

In our analysis NO₂ was the pollutant with the most consistent effect. NO₂ has been suggested to be a good marker for exposure with UFPs (diameter < 1 μ m) (Seaton and Dennekamp, 2003; World Health Organization, 2006). To date toxicological and epidemiological studies have not provided consistent findings on the health effects of exposures to ambient levels of UFPs. However, compared with larger particles UFPs deposit with higher efficiency, are cleared more slowly and might be able to translocate into the systemic circulation (HEI 2013).

4.2. Whole blood assay as a promising tool in epidemiological studies

Whole blood assays, like the one used in the present study, have been performed mostly with lipopolysaccharide (LPS) endotoxin as stimulus (Herberth et al., 2009) and the molecular mechanisms whereby LPS triggers inflammatory signal cascades are well known (Hasiwa et al., 2013). However, as we wanted to assess the effect of traffic-related exposure we chose EHC-93 as stimulus. Even though EHC-93 is a several decade old urban dust, that is probably not representative for the air pollution the children in our study were exposed to, we have chosen this stimulus as it is a well-known PM-reference with known elemental composition and pro-inflammatory potency (Vincent et al., 1997) and available in sufficient quantity for large scale studies. However, future studies should investigate the inflammatory response of PM samples collected at the study sites that are representative of the air pollution exposure at these sites.

We used a dose of 100 μ g/ml selected on the basis of dose-response studies with blood samples from healthy donors. Obviously, the dose in this WBA by far exceeds concentrations of PM or its specific components (e.g. metals) that might reach the blood after inhalation. The choice of the dose used in the WBA was made on its ability to detect contrasting responsiveness between blood samples from different individuals. However, findings have to be interpreted with caution and the degree to which the results can be generalized to real life situations is limited. EHC-93 particles contain small amounts of endotoxin (Vincent et al., 1997). The particle dose used in our study corresponds to only trace amounts (< 2ng) of endotoxin (van Eeden et al., 2001) that are unlikely to cause the responsiveness of certain inflammatory markers observed in our study. However, previous investigations with coarse and fine PM collected from urban versus rural environment suggest a contribution of endotoxin to cytokine release in whole blood assays (Schins et al., 2004) and we cannot fully exclude that residues of endotoxin in EHC-93 particles are partly responsible for the observed cytokine release.

Using air-liquid interface cultures from asthmatic and non-asthmatic epithelial cells Hackett et al. (2011) have recently shown an aberrant IL-6 and IL-8 response in asthmatic epithelium after exposure to EHC-93 (Hackett et al., 2011). However, in contrast to our findings there was no significant production of TNF- α after challenge with EHC-93. As epithelial cells do not produce considerable amounts of TNF- α results are not conflicting. We observed that EHC-93 is highly potent in inducing the production of the pro-inflammatory cytokines IL-6, IL-8 and TNF- α in whole blood. The production of the anti-inflammatory cytokine IL-10 and the chemokine MCP-1 was stimulated to a lesser extent and after stimulation there was no production of IL-12p70 and IFN- γ . IL-6, IL-8 and TNF- α are mainly produced by monocytes and macrophages. Uptake of particles by phagocytosis stimulates these cells to produce pro-inflammatory markers.

T-lymphocytes and natural killer cells producing INF-gamma are not directly stimulated by particles. Thus, it is plausible that the production of IFN-gamma is not stimulated by EHC-93 in the whole blood assay. Our results are in concordance with findings obtained with diesel engine particles in dendritic cells (Ohtani et al., 2005; Williams et al., 2007) showing that the production of IL-12 and IFN-gamma is not stimulated by urban dust.

4.3. Strengths and limitations

So far, this is the first study assessing the effect of long-term TRAP exposure on inflammatory patterns by using a WBA. Compared to measurements of instantaneous levels of cytokines whole blood cytokine responsiveness is more independent of short-term effects influencing the cytokine level at the time of sampling. Furthermore, using the WBA is advantageous because specific inflammatory responses can be measured in the presence of autologous plasma components and cross talk between different cell types or subsets of cell types is still possible whereas artifacts due to isolation of cells can be excluded (Schins et al., 1996).

A major strength of our study is the assessment of TRAP exposure on the residential level by using the validated ESCAPE exposure modeling. As we wanted to investigate potential effect modifications of asthma status in the association between long-term TRAP exposure and whole blood cytokine responsiveness using a study population enriched with asthmatic children is also advantageous.

The main limitation of our study is the comparatively small sample size. Therefore, results have to be interpreted cautiously and we might not have sufficient power to detect smaller differences in cytokine production patterns between asthmatic and non-asthmatic children or to detect a much more moderate association between TRAP exposure and pro-inflammatory response in non-asthmatic children. The proportion of asthmatic children with anti-inflammatory asthma medication is relatively high in our study (almost 50%) and we cannot fully exclude that this has biased our results. However, excluding children with anti-inflammatory asthma medication had little impact on our results.

As we were mainly interested in the effect of long-term TRAP exposure on cytokine responsiveness we did not investigate the effect of short-term exposure. However, short-term effects might also be relevant and should be studied in future studies.

The long-term air pollution estimates were derived from LUR models built using data from the ESCAPE measurement campaign between 2008 and 2009. As measurements were done after blood sampling campaign between 2002 and 2005 we investigated the validity of the modeled exposure estimates by applying the ESCAPE land-use regression models to air pollution measurements done earlier in 2002 and 2003 (Hochadel et al., 2006). We calculated the annual means using these earlier measurements and compared them with annual means from ESCAPE models. The correlation coefficients of the concentration measurements from 2002/2003 and the estimates using ESCAPE measurements were 0.9 for NO₂, 0.8 for PM_{2.5abs} and 0.3 for PM_{2.5mass}. Additionally, we looked at the annual mean concentrations of air pollutants (PM₁₀, NO₂ and NO_x) measured between 2002 and 2009 at the local governmental monitoring station (see table S6) nearby the study center Wesel and found no statistically significant trend. Furthermore, LUR models for NO₂ have been shown to be temporally stable over 7–12 years (Cesaroni et al. 2012; Eeftens et al. 2011; Wang et al. 2013). Thus, it is reasonable to assume that air pollution patterns remained stable between the year of blood sampling and the ESCAPE measurement campaign.

In this study asthma cases were identified on the basis of a physical examination by a pediatric pulmonologist. We were not able to discriminate between different asthma phenotypes that

might be related to different inflammatory patterns in peripheral blood. A more differentiated case classification with regard to inflammatory markers would be an interesting approach for future research.

5. Conclusion

Our results suggest an effect modification by asthma status for the association of long-term residential TRAP exposure with whole blood pro-inflammatory cytokine responsiveness in six-year-old children. Thus, different systemic pro-inflammatory response patterns might be the key to understand the higher susceptibility of asthmatic individuals. The WBA seems to be an useful and simple tool for epidemiological studies to gain better knowledge on the effect of TRAP exposure and cytokine production patterns. Future large-scale studies should attempt to replicate our findings.

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Contributor Statement

DB, AvB, JH, BH, UK, IL and RPFS were responsible for the design, hypothesis delineation and conception of the study. CB, DB, AvB, JH, GH, UK, IL, EL and RPFS contributed to the data collection and data management. CK and UK contributed to statistical analysis. CK wrote the manuscript; all other authors critically reviewed the manuscript and approved the final version of the manuscript for submission.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2015.02.034>.

References

- Andersen, Z.J., Bonnelykke, K., Hvidberg, M., Jensen, S.S., Ketzel, M., Loft, S., Sørensen, M., Tjønneland, A., Overvad, K., Raaschou-Nielsen, O., 2012. Long-term exposure to air pollution and asthma hospitalisations in older adults: a cohort study. *Thorax* 67, 6–11.
- Auerbach, A., Hernandez, M.L., 2012. The effect of environmental oxidative stress on airway inflammation. *Curr Opin Allergy Clin Immunol* 12, 133–139.
- Baines, K.J., Simpson, J.L., Wood, L.G., Scott, R.J., Gibson, P.G., 2011. Systemic up-regulation of neutrophil α -defensins and serine proteases in neutrophilic asthma. *Thorax* 66, 942–947.
- Balmes, J.R., 2011. How does diesel exhaust impact asthma? *Thorax* 66, 4–6.
- Barraza-Villarreal, A., Sunyer, J., Hernandez-Cadena, L., Escamilla-Núñez, M.C., Sierra-Monge, J.J., Ramírez-Aguilar, M., Cortez-Lugo, M., Holguin, F., Díaz-Sánchez, D., Olin, A.C., Romieu, I., 2008. Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. *Environ Health Perspect* 116, 832–838.
- Behndig, A.F., Larsson, N., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Dove, R. E., Wilson, S.J., Sandstrom, T., Kelly, F.J., Mudway, I.S., Blomberg, A., 2011. Proinflammatory doses of diesel exhaust in healthy subjects fail to elicit equivalent or augmented airway inflammation in subjects with asthma. *Thorax* 66, 12–19.
- Brauer, M., Hoek, G., Smit, H.A., de Jongste, J.C., Gerritsen, J., Postma, D.J., Kerkhof, M., Brunekreef, B., 2007. Air pollution and development of asthma, allergy and infections in a birth cohort. *Eur Respir J* 29, 879–888.
- Braun-Fahrlander, C., Vuille, J.C., Sennhauser, F.H., Neu, U., Künzle, T., Grize, L., Gassner, M., Minder, C., Schindler, C., Varonier, H.S., Wüthrich, B., 1997. Respiratory health and long-term exposure to air pollutants in Swiss schoolchildren. SCARPOL Team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution, Climate and Pollen. *Am J Respir Crit Care Med* 155 (3), 1042–1049.
- Brightling, C., Berry, M., Amrani, Y., 2008. Targeting TNF- α : a novel therapeutic approach for asthma. *J Allergy Clin Immunol* 121 5–10, 11–12, quiz.
- Brown, M.S., Sarnat, S.E., DeMuth, K.A., Brown, L.A.S., Whitlock, D.R., Brown, S.W., Tolbert, P.E., Fitzpatrick, A.M., 2012. Residential proximity to a major roadway is associated with features of asthma control in children. *PLoS ONE* 7, e37044.
- Cesaroni, G., Porta, D., Badaloni, C., Stafoggia, M., Eeftens, M., Meliefste, K., Forastiere, F., 2012. Nitrogen dioxide levels estimated from land use regression models several years apart and association with mortality in a large cohort study. *Environ. Health* 11, 48–57.
- Chauhan, A.J., Johnston, S.L., 2003. Air pollution and infection in respiratory illness. *British Medical Bulletin* 68, 95–112.
- Cyrys, J., Eeftens, M., Heinrich, J., Ampe, C., Armengaud, A., Beelen, R., Bellander, T., Beregszaszi, T., Birk, M., Cesaroni, G., Cirach, M., de Hoogh, K., De Nazelle, A., de Vocht, F., Declercq, C., Dedele, A., Dimakopoulou, K., Eriksen, K., Galassi, C., Graulén, R., Grivas, G., Gruzdeva, O., Gustafsson, A.H., Hoffmann, B., Iakovides, M., Ineichen, A., Krämer, U., Lanki, T., Lozano, P., Madsen, C., Meliefste, K., Modig, L., Mölter, A., Mosler, G., Nieuwenhuijsen, M., Nonnemacher, M., Oldenwening, M., Peters, A., Pontet, S., Probst-Hensch, N., Quass, U., Raaschou-Nielsen, O., Ranzi, A., Sugiri, D., Stephanou, E.G., Taimisto, P., Tsai, M.-Y., Vaskövi, É., Villani, S., Wang, M., Brunekreef, B., Hoek, G., 2012. Variation of NO₂ and NO_x concentrations between and within 36 European study areas: Results from the ESCAPE study. *Atmospheric Environment* 62, 374–390.
- Eeftens, M., Beelen, R., Fischer, P., Brunekreef, B., Meliefste, K., Hoek, G., 2011. Stability of measured and modelled spatial contrasts in NO₂ over time. *Occup Environ Med* 68, 765–770.
- Eeftens, M., Beelen, R., de Hoogh, K., Bellander, T., Cesaroni, G., Cirach, M., Declercq, C., Dedele, A., Dons, E., de Nazelle, A., Dimakopoulou, K., Eriksen, K., Falq, G., Fischer, P., Galassi, C., Graulén, R., Heinrich, J., Hoffmann, B., Jerrett, M., Keidel, D., Korek, M., Lanki, T., Lindley, S., Madsen, C., Mölter, A., Nádor, G., Nieuwenhuijsen, M., Nonnemacher, M., Pedeli, X., Raaschou-Nielsen, O., Paterlarou, E., Quass, U., Ranzi, A., Schindler, C., Stempfelet, M., Stephanou, E., Sugiri, D., Tsai, M.-Y., Yli-Tuomi, T., Varró, M.J., Vienneau, D., Klot, S., von Wolf, K., Brunekreef, B., Hoek, G., 2012. Development of Land Use Regression Models for PM_{2.5}, PM_{2.5} Absorbance, PM₁₀ and PM_{coarse} in 20 European Study Areas: Results of the ESCAPE Project. *Environ Sci Technol* 46, 11195–11205.
- Gauderman, W.J., Avol, E., Lurmann, F., Kuenzli, N., Gilliland, F., Peters, J., McConnell, R., 2005. Childhood Asthma and Exposure to Traffic and Nitrogen Dioxide. *Epidemiology* 16, 737–743.
- Hackett, T.-L., Singhera, G.K., Shaheen, F., Hayden, P., Jackson, G.R., Hegele, R.G., Van Eeden, S., Bai, T.R., Dorscheid, D.R., Knight, D.A., 2011. Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 45, 1090–1100.
- Hasiwa, N., Daneshian, M., Bruegger, P., Fennrich, S., Hochadel, A., Hoffmann, S., Rivera-Mariani, F.E., Rockel, C., Schindler, S., Spreitzer, I., Stoppelkamp, S., Vysaraju, K., Hartung, T., 2013. Evidence for the detection of non-endotoxin pyrogens by the whole blood monocyte activation test. *ALTEX* 30, 169–208.
- Health Effects Institute, 2010. Traffic-related Air Pollution: A Critical Review of the Literature on Emissions, Exposures, and Health Effects-Special Report, 17.
- HEI Review Panel on Ultrafine Particles. 2013. Understanding the Health Effects of Ambient Ultrafine Particles. HEI Perspectives 3. Health Effects Institute, Boston, MA.
- Heinrich, J., Hoelscher, B., Wichmann, H.E., 2000. Decline of ambient air pollution and respiratory symptoms in children. *Am J Respir Crit Care Med* 161 (6), 1930–1936.
- Herberth, G., Gubelt, R., Röder, S., Krämer, U., Schins, R.P.F., Diez, U., Borte, M., Heinrich, J., Wichmann, H.-E., Herbarth, O., Lehmann, I., 2009. Increase of inflammatory markers after indoor renovation activities: the LISA birth cohort study. *Pediatr Allergy Immunol* 20, 563–570.
- Herberth, G., Weber, A., Röder, S., Elvers, H.-D., Krämer, U., Schins, R.P.F., Diez, U., Borte, M., Heinrich, J., Schäfer, T., Herbarth, O., Lehmann, I., 2008. Relation between stressful life events, neuropeptides and cytokines: results from the LISA birth cohort study. *Pediatr Allergy Immunol* 19, 722–729.
- Hochadel, M., Heinrich, J., Gehring, U., Morgenstern, V., Kuhlbusch, T., Link, E., Wichmann, H.-E., Krämer, U., 2006. Predicting long-term average concentrations of traffic-related air pollutants using GIS-based information. *Atmospheric Environment* 40, 542–553.
- Johnston, N.W., Sears, M.R., 2006. Asthma exacerbations. *Thorax* 61, 722–728.
- Künzli, N., Perez, L., Lurmann, F., Hricko, A., Penfold, B., McConnell, R., 2008. An attributable risk model for exposures assumed to cause both chronic disease and its exacerbations. *Epidemiology* 19 (2), 179–185.
- Mann, J.K., Balmes, J.R., Bruckner, T.A., Mortimer, K.M., Margolis, H.G., Pratt, B., Hammond, S.K., Lurmann, F.W., Trager, I.B., 2010. Short-Term Effects of Air Pollution on Wheeze in Asthmatic Children in Fresno, California. *Environ Health Perspect* 118, 1497–1502.
- McConnell, R., Berhane, K., Gilliland, F., London, S.J., Vora, H., Avol, E., Gauderman, W.J., Margolis, H.G., Lurmann, F., Thomas, D.C., Peters, J.M., 1999. Air pollution and bronchitic symptoms in Southern California children with asthma. *Environ Health Perspect* 107 (9), 757–760.
- McConnell, R., Berhane, K., Gilliland, F., Molitor, J., Thomas, D., Lurmann, F., Avol, E., Gauderman, W.J., Peters, J.M., 2003. Prospective study of air pollution and bronchitic symptoms in children with asthma. *Am J Respir Crit Care Med* 168 (7), 790–797.
- Nadif, R., Siroux, V., Oryszczyn, M.-P., Ravault, C., Pison, C., Pin, I., Kauffmann, F., 2009. Heterogeneity of asthma according to blood inflammatory patterns. *Thorax* 64, 374–380.
- Nakane, H., 2012. Translocation of particles deposited in the respiratory system: a systematic review and statistical analysis. *Environ. Health Prev Med* 17, 263–274.
- Nemmar, A., Holme, J.A., Rosas, I., Schwarze, P.E., Alfaro-Moreno, E., 2013. Recent advances in particulate matter and nanoparticle toxicology: a review of the in vivo and in vitro studies. *Biomed Res Int* 2013, 279371.
- Neurath, M.F., Finotto, S., 2011. IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev* 22, 83–89.
- Ohtani, T., Nakagawa, S., Kurosawa, M., Mizuashi, M., Ozawa, M., Aiba, S., 2005. Cellular basis of the role of diesel exhaust particles in inducing Th2-dominant response. *J Immunol* 174, 2412–2419.
- Schins, R.P., van Hartingsveldt, B., Borm, P.J., 1996. Ex vivo cytokine release from whole blood. A routine method for health effect screening. *Exp Toxicol Pathol* 48, 494–496.
- Schins, R.P.F., Lightbody, J.H., Borm, P.J.A., Shi, T., Donaldson, K., Stone, V., 2004. Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents. *Toxicol Appl Pharmacol* 195, 1–11.
- Seaton, A., Dennekamp, M., 2003. Hypothesis: Ill health associated with low concentrations of nitrogen dioxide — an effect of ultrafine particles? *Thorax* 58, 1012–1015.
- Segala, C., Fauroux, B., Just, J., Pascual, L., Grimfeld, A., Neukirch, F., 1998. Short-term effect of winter air pollution on respiratory health of asthmatic children in Paris. *Eur Respir J* 11 (3), 677–685.
- Sennhauser, F.H., Braun-Fahrlander, C., Wildhaber, J.H., 2005. The burden of asthma in children: a European perspective. *Paediatr Respir Rev* 6 (1), 2–7.
- Stenfors, N., Nordenhäll, C., Salvi, S.S., Mudway, I., Söderberg, M., Blomberg, A., Helleday, R., Levin, J.O., Holgate, S.T., Kelly, F.J., Frew, A.J., Sandström, T., 2004. Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. *Eur Respir J* 23, 82–86.
- Van Eeden, S.F., Tan, W.C., Suwa, T., Mukae, H., Terashima, T., Fujii, T., Qui, D., Vincent, R., Hogg, J.C., 2001. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM₁₀). *Am J Respir Crit Care Med* 164, 826–830.
- Vincent, R., Bjarnason, S.G., Adamson, I.Y., Hedgecock, C., Kumarathasan, P., Guénette, J., Potvin, M., Goegan, P., Bouthillier, L., 1997. Acute pulmonary toxicity of urban particulate matter and ozone. *Am J Pathol* 151, 1563–1570.
- Wang, R., Henderson, S.B., Sbihi, H., Allen, R.W., Brauer, M., 2013. Temporal stability

- of land use regression models for traffic-related air pollution. *Atmos Environ* 64, 312–319.
- Wessels, A., Birmili, W., Albrecht, C., Hellack, B., Jermann, E., Wick, G., Harrison, R. M., Schins, R.P.F., 2010. Oxidant generation and toxicity of size-fractionated ambient particles in human lung epithelial cells. *Environ Sci Technol* 44, 3539–3545.
- World Health Organization. 2006. WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide, and sulfur dioxide. Global Update 2005.
- Williams, M.A., Porter, M., Horton, M., Guo, J., Roman, J., Williams, D., Breyse, P., Georas, S.N., 2007. Ambient particulate matter directs nonclassic dendritic cell activation and a mixed TH1/TH2-like cytokine response by naive CD4+ T cells. *J. Allergy Clin. Immunol.* 119, 488–497.
- Wouters, E.F.M., Reynaert, N.L., Dentener, M.A., Vernooij, J.H.J., 2009. Systemic and local inflammation in asthma and chronic obstructive pulmonary disease: is there a connection? *Proc Am Thorac Soc* 6, 638–647.
- Yeatts, K., Svendsen, E., Creason, J., Alexis, N., Herbst, M., Scott, J., Kupper, L., Williams, R., Neas, L., Cascio, W., Devlin, R.B., Peden, D.B., 2007. Coarse particulate matter (PM_{2.5-10}) affects heart rate variability, blood lipids, and circulating eosinophils in adults with asthma. *Environ Health Perspect* 115, 709–714.
- Zirngibl, A., Franke, K., Gehring, U., von Berg, A., Berdel, D., Bauer, C.P., Reinhardt, D., Wichmann, H.-E., Heinrich, J., 2002. Exposure to pets and atopic dermatitis during the first two years of life. A cohort study. *Pediatr Allergy Immunol* 13, 394–401.
- Zutavern, A., Brockow, I., Schaaf, B., Bolte, G., von Berg, A., Diez, U., Borte, M., Herbarth, O., Wichmann, H.-E., Heinrich, J., 2006. Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics* 117, 401–411.

Supplementary File

Whole blood assay (WBA)

Heparinized blood samples (5 ml) from children were processed within 4 h. For cytokine analyses in spontaneous blood samples (SP tubes), 0.5 ml heparinized blood was diluted 1:1 with RPMI-1640 medium without supplements in endotoxin free polypropylene tubes. Ex-vivo stimulation of blood samples with particulate matter (PM tubes) was performed by using EHC-93 urban dust at a final concentration of 100 µg/ml in the RPMI-diluted blood. The concentration of 100 µg/ml EHC-93 was chosen on the basis of pilot experiments in a wide concentrations range in which dose-response curves for TNF-alpha and IL-8 were evaluated in relation to cell toxicity as well as absorption effects (data not shown). The SP and PM tubes were incubated for 4 h at 37° C on a gently shaking thermoblock and then centrifugated at 800 g for 5 min at 4° C. The supernatants free of cells and particles were collected and stored below -20° C until subsequent cytokine measurement.

Statistical Analyses

To prove whether long-term traffic-related air pollution exposure has an effect on cytokine responsiveness in the overall group we applied the following crude linear regression model:

Model 1:

$$\log\left(\frac{\text{Cytokine}_{\text{stimulated}}}{\text{Cytokine}_{\text{non-stimulated}}}\right) = \alpha + \beta_1 \times \text{pollutant}$$

To prove our research hypothesis that long-term traffic-related air pollution exposure has different effects on the blood responsiveness in asthmatic and non-asthmatic

children and to evaluate effect modification by asthma status we used a model including air pollution exposure and asthma status as independent variables and a multiplicative interaction term between air pollution exposure and asthma status. Based upon the estimate for the main effect (β_1) and the interaction term (β_3) we calculated stratum-specific effect-estimates: The effect estimate for the non-asthmatics is β_1 and the effect estimate for the asthmatics is $\beta_1 + \beta_3$.

Model 2:

$$\log\left(\frac{\text{Cytokine}_{\text{stimulated}}}{\text{Cytokine}_{\text{non-stimulated}}}\right) = \alpha + \beta_1 \times \text{pollutant} + \beta_2 \times \text{asthma} + \beta_3 \times (\text{pollutant} \times \text{asthma})$$

All models were run as crude (see formulas above) and adjusted models. Potential confounders were identified based upon knowledge from our previous studies and knowledge from the literature. Data on potential confounders were obtained from parental questionnaires. We used two different adjustment sets (main model and extended). For our main model we additionally included the covariates asthma status (only model 1) and sex in the crude model. In the extended model the following 7 covariates were included: asthma status (only model 1), sex, parental education (categorized as <10 years, =10 years and >10 years), siblings, current environmental tobacco smoke at child's home, visible signs of mold or dampness in the home and body mass index of the child at the age of 6. Except for parental education (categorical variable) and body mass index all covariates were included as binary variables.

Parental allergy was not included in our main and extended model as it is highly correlated with asthma status and thus might lead to over-adjustment. However, parental allergy was considered in sensitivity analyses. Day-care center attendance within the first 2 years of life was also not included in the models as none of the children visited daycare centers. We performed additional sensitivity analyses by separately

including the following variables in our main model: parental allergy, age at blood sampling, season of blood sampling, time between blood sampling and start of incubation, incubation duration as well as time between the end of incubation and freezing. Except for parental allergy (binary variable) and season of blood sampling (categorical variable) all covariates were included as continuous variables.

Table S1 Blood responsiveness in asthmatic and non-asthmatic children: Ratios PM-stimulated/spontaneous blood. Values are presented medians with interquartile ranges (IQR)

Ratio: PM-stimulated/spontaneous blood						
	All children			Children without medication		
	Asthma + (N=27)	Asthma – (N=59)		Asthma + (N=8)	Asthma – (N=58)	
	Median (IQR)	Median (IQR)	p*	Median (IQR)	Median (IQR)	p*
IL-6	231.4 (149.7-490.9)	363.1 (156.2-556.7)	0.5894	217.7 (166.5-536.2)	336.0 (156.2-556.7)	0.8136
IL-8	63.8 (25.9-133.9)	101.0 (39.9-155.4)	0.3868	69.2 (38.2-225.7)	98.1 (39.1-155.4)	0.8519
IL-10	5.6 (4.2-7.4)	4.6 (2.6-7.2)	0.1136	7.3 (4.7-11.4)	4.6 (2.6-7.2)	0.0529
IL-12p70	0.5 (0.4-1.0)	0.6 (0.4-1.0)	0.7300	0.7 (0.5-1.0)	0.6 (0.4-1.0)	0.5546
MCP1	6.1 (3.8-11.5)	6.1 (2.8-10.3)	0.6551	6.1 (4.5-20.0)	6.1 (2.8-10.3)	0.3928
TNF α	132.5 (75.5-304.4)	142.3 (45.0-221.4)	0.3920	189.2 (123.8-368.7)	149.9 (45.0-221.3)	0.1230
IFN- gamma	0.4 (0.3-1.0)	0.6 (0.2-1.0)	0.5147	0.7 (0.2-2.1)	0.6 (0.2-1.0)	0.7666

*p values for differences between children with and without asthma using Mann-Whitney U-Test

Table S2 Effect of traffic air pollution (MR: Means ratio) on whole blood cytokine responsiveness - Results of model 1

	Model 1 crude (N=86)		Model 1 main¹ (N=86)		Model 1 extended² (N=81)	
	MR (95% CI)	p-value	MR (95% CI)	p-value	MR (95% CI)	p-value
IL-6						
NO₂	0.97 (0.77-1.22)	0.794	0.99 (0.79-1.25)	0.944	0.95 (0.75-1.21)	0.688
NO_x	0.96 (0.80-1.16)	0.693	0.98 (0.82-1.18)	0.840	0.95 (0.79-1.16)	0.629
PM_{2.5mass}	0.97 (0.68-1.38)	0.875	0.99 (0.70-1.40)	0.974	1.01 (0.69-1.46)	0.971
PM₁₀	1.25 (0.92-1.69)	0.153	1.31 (0.97-1.77)	0.077	1.25 (0.90-1.73)	0.176
PM_{coarse}	0.99 (0.78-1.27)	0.954	1.04 (0.82-1.34)	0.734	1.02 (0.79-1.32)	0.864
PM_{2.5abs}	1.21 (0.89-1.64)	0.227	1.29 (0.95-1.75)	0.104	1.22 (0.88-1.69)	0.242
IL-8						
NO₂	0.81 (0.63-1.05)	0.109	0.81 (0.63-1.05)	0.115	0.79 (0.60-1.05)	0.104
NO_x	0.84 (0.69-1.03)	0.099	0.84 (0.69-1.04)	0.107	0.83 (0.67-1.04)	0.103
PM_{2.5mass}	0.86 (0.58-1.28)	0.467	0.87 (0.58-1.28)	0.472	0.92 (0.60-1.41)	0.690
PM₁₀	1.09 (0.77-1.53)	0.635	1.10 (0.78-1.56)	0.596	1.15 (0.79-1.68)	0.470
PM_{coarse}	0.91 (0.69-1.20)	0.498	0.91 (0.69-1.21)	0.532	0.93 (0.69-1.25)	0.629
PM_{2.5abs}	1.03 (0.73-1.45)	0.884	1.04 (0.73-1.48)	0.822	1.07 (0.73-1.57)	0.734
IL-10						
NO₂	1.05 (0.89-1.23)	0.579	1.08 (0.92-1.25)	0.345	1.05 (0.89-1.25)	0.546
NO_x	1.02 (0.90-1.16)	0.712	1.05 (0.93-1.18)	0.461	1.03 (0.90-1.18)	0.677
PM_{2.5mass}	0.94 (0.74-1.20)	0.616	0.97 (0.77-1.22)	0.797	0.93 (0.72-1.20)	0.569
PM₁₀	0.96 (0.77-1.18)	0.677	1.01 (0.82-1.24)	0.940	0.94 (0.75-1.18)	0.599
PM_{coarse}	0.99 (0.84-1.18)	0.952	1.06 (0.90-1.25)	0.488	1.04 (0.87-1.24)	0.678
PM_{2.5abs}	0.98 (0.80-1.22)	0.875	1.05 (0.85-1.29)	0.643	0.98 (0.78-1.23)	0.853

IL-12p70						
NO₂	1.01 (0.89-1.15)	0.851	1.01 (0.89-1.14)	0.933	0.98 (0.87-1.10)	0.728
NO_x	1.01 (0.91-1.11)	0.881	1.00 (0.91-1.11)	0.965	0.98 (0.89-1.08)	0.681
PM_{2.5mass}	1.00 (0.83-1.21)	0.959	1.00 (0.83-1.20)	0.980	0.98 (0.82-1.18)	0.860
PM₁₀	1.00 (0.85-1.18)	0.997	0.99 (0.84-1.17)	0.884	0.97 (0.82-1.14)	0.705
PM_{coarse}	1.01 (0.88-1.15)	0.915	0.99 (0.87-1.14)	0.911	1.01 (0.89-1.14)	0.921
PM_{2.5abs}	1.01 (0.85-1.19)	0.948	0.99 (0.84-1.17)	0.905	0.97 (0.82-1.14)	0.686
MCP-1						
NO₂	0.78 (0.60-1.00)	0.053	0.78 (0.60-1.01)	0.055	0.77 (0.59-1.00)	0.050
NO_x	0.81 (0.66-0.99)	0.042	0.81 (0.66-0.99)	0.043	0.80 (0.65-0.99)	0.039
PM_{2.5mass}	0.81 (0.55-1.20)	0.301	0.81 (0.55-1.21)	0.311	0.83 (0.55-1.26)	0.387
PM₁₀	0.93 (0.66-1.32)	0.695	0.94 (0.66-1.34)	0.727	0.92 (0.63-1.32)	0.639
PM_{coarse}	0.92 (0.70-1.21)	0.566	0.93 (0.70-1.23)	0.598	0.90 (0.68-1.20)	0.486
PM_{2.5abs}	0.92 (0.65-1.30)	0.629	0.92 (0.65-1.32)	0.661	0.89 (0.61-1.28)	0.519
TNF-alpha						
NO₂	1.02 (0.84-1.25)	0.818	1.05 (0.86-1.28)	0.664	1.04 (0.83-1.29)	0.758
NO_x	1.01 (0.86-1.19)	0.893	1.03 (0.88-1.21)	0.744	1.02 (0.86-1.21)	0.832
PM_{2.5mass}	0.91 (0.67-1.23)	0.54	0.93 (0.69-1.26)	0.643	0.96 (0.69-1.34)	0.813
PM₁₀	1.08 (0.83-1.41)	0.586	1.13 (0.86-1.47)	0.385	1.17 (0.87-1.57)	0.305
PM_{coarse}	1.02 (0.83-1.27)	0.828	1.08 (0.87-1.34)	0.507	1.12 (0.89-1.41)	0.338
PM_{2.5abs}	1.06 (0.81-1.38)	0.689	1.11 (0.85-1.46)	0.435	1.16 (0.86-1.56)	0.338
IFN-gamma						
NO₂	0.96 (0.77-1.18)	0.680	0.96 (0.78-1.19)	0.719	0.94 (0.74-1.18)	0.580
NO_x	0.95 (0.81-1.13)	0.588	0.96 (0.81-1.14)	0.622	0.94 (0.78-1.12)	0.480
PM_{2.5mass}	0.95 (0.69-1.32)	0.771	0.96 (0.69-1.33)	0.804	0.89 (0.63-1.26)	0.515
PM₁₀	0.89 (0.67-1.17)	0.395	0.89 (0.67-1.19)	0.433	0.82 (0.60-1.12)	0.208
PM_{coarse}	0.97 (0.78-1.22)	0.804	0.98 (0.78-1.24)	0.891	0.92 (0.72-1.17)	0.504
PM_{2.5abs}	0.94 (0.71-1.25)	0.663	0.95 (0.71-1.27)	0.729	0.89 (0.65-1.22)	0.468

¹Adjusted for asthma status, gender

²Adjusted for sex, parental education, siblings, current environmental tobacco smoke at child's home, visible signs of mold or dampness in the home, body mass index

Table S3 Stratum specific effect estimates¹ (MR: Means ratio) of traffic-related air pollution exposure on whole blood cytokine responsiveness in asthmatic and non-asthmatic children – results based on model with interaction term

	Model 2 crude (N=86)		Model 2 main ² (N=86)		Model 2 extended ³ (N=81)	
	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value
IL-6						
NO₂	2.22 (1.22-4.04) 0.009	0.86 (0.67-1.09) 0.199	2.16 (1.20-3.90) 0.010	0.87 (0.69-1.11) 0.263	2.24 (1.23-4.09) 0.009	0.84 (0.66-1.07) 0.158
NO_x	1.86 (1.16-2.97) 0.009	0.87 (0.72-1.05) 0.145	1.82 (1.15-2.88) 0.011	0.88 (0.73-1.07) 0.201	1.88 (1.17-3.01) 0.009	0.86 (0.71-1.04) 0.127
PM_{2.5mass}	1.88 (0.85-4.18) 0.120	0.84 (0.57-1.24) 0.381	1.89 (0.87-4.12) 0.109	0.86 (0.59-1.25) 0.418	2.04 (0.94-4.42) 0.070	0.83 (0.55-1.25) 0.365
PM₁₀	1.99 (0.99-3.99) 0.053	1.15 (0.82-1.61) 0.422	2.04 (1.03-4.02) 0.039	1.18 (0.85-1.64) 0.320	1.99 (0.96-4.12) 0.063	1.13 (0.79-1.60) 0.509
PM_{coarse}	1.17 (0.62-2.20) 0.624	0.98 (0.75-1.29) 0.879	1.22 (0.66-2.26) 0.534	1.01 (0.78-1.33) 0.916	1.12 (0.59-2.12) 0.729	1.01 (0.76-1.33) 0.962
PM_{2.5aabs}	2.10 (0.96-4.58) 0.062	1.12 (0.80-1.57) 0.502	2.23 (1.05-4.76) 0.038	1.16 (0.84-1.61) 0.359	1.98 (0.90-4.35) 0.090	1.11 (0.78-1.58) 0.554
IL-8						
NO₂	1.50 (0.76-2.97) 0.243	0.73 (0.56-0.96) 0.023	1.48 (0.75-2.93) 0.256	0.74 (0.56-0.97) 0.028	1.67 (0.83-3.38) 0.153	0.71 (0.53-0.95) 0.020
NO_x	1.39 (0.81-2.36) 0.230	0.77 (0.62-0.96) 0.019	1.37 (0.81-2.33) 0.245	0.78 (0.63-0.97) 0.024	1.52 (0.87-2.63) 0.138	0.76 (0.60-0.95) 0.018
PM_{2.5mass}	1.87 (0.76-4.57) 0.170	0.72 (0.47-1.10) 0.129	1.87 (0.77-4.56) 0.167	0.72 (0.47-1.11) 0.138	1.98 (0.81-4.86) 0.136	0.74 (0.46-1.19) 0.214
PM₁₀	2.04 (0.92-4.49) 0.078	0.93 (0.64-1.37) 0.721	2.07 (0.94-4.53) 0.070	0.95 (0.65-1.39) 0.784	2.43 (1.05-5.64) 0.038	0.97 (0.64-1.46) 0.887
PM_{coarse}	1.01 (0.50-2.06) 0.968	0.88 (0.65-1.19) 0.399	1.03 (0.51-2.10) 0.924	0.89 (0.66-1.21) 0.470	0.98 (0.47-2.06) 0.962	0.92 (0.67-1.27) 0.611
PM_{2.5aabs}	1.59 (0.65-3.88) 0.308	0.94 (0.64-1.38) 0.744	1.65 (0.68-4.00) 0.272	0.96 (0.65-1.40) 0.827	1.74 (0.69-4.39) 0.241	0.98 (0.65-1.47) 0.911
IL-10						
NO₂	1.11 (0.73-1.70) 0.621	1.05 (0.89-1.25) 0.546	1.09 (0.72-1.64) 0.687	1.07 (0.91-1.27) 0.393	1.06 (0.69-1.65) 0.787	1.05 (0.88-1.26) 0.580
NO_x	1.09 (0.78-1.52) 0.622	1.03 (0.90-1.18) 0.710	1.06 (0.77-1.47) 0.706	1.04 (0.91-1.19) 0.522	1.05 (0.74-1.48) 0.798	1.03 (0.89-1.18) 0.721

PM_{2.5mass}	0.97 (0.56-1.68) 0.902	0.96 (0.73-1.25) 0.750	0.97 (0.57-1.66) 0.907	0.97 (0.75-1.26) 0.819	0.97 (0.56-1.67) 0.909	0.92 (0.69-1.22) 0.559
PM₁₀	0.76 (0.47-1.23) 0.264	1.05 (0.83-1.33) 0.682	0.77 (0.48-1.23) 0.282	1.07 (0.85-1.35) 0.546	0.71 (0.43-1.18) 0.182	1.00 (0.78-1.29) 0.977
PM_{coarse}	0.90 (0.59-1.38) 0.627	1.06 (0.88-1.27) 0.555	0.93 (0.61-1.40) 0.718	1.09 (0.91-1.30) 0.361	0.88 (0.57-1.37) 0.576	1.07 (0.88-1.29) 0.497
PM_{2.5abs}	0.68 (0.40-1.16) 0.157	1.10 (0.87-1.38) 0.421	0.71 (0.42-1.19) 0.195	1.13 (0.90-1.41) 0.288	0.63 (0.36-1.08) 0.093	1.06 (0.83-1.35) 0.624
IL-12p70						
NO₂	1.08 (0.77-1.51) 0.657	1.00 (0.87-1.14) 0.983	1.09 (0.78-1.52) 0.628	0.99 (0.87-1.14) 0.916	0.98 (0.71-1.34) 0.889	0.98 (0.86-1.11) 0.745
NO_x	1.06 (0.81-1.38) 0.672	1.00 (0.90-1.11) 0.959	1.07 (0.82-1.38) 0.636	0.99 (0.89-1.10) 0.883	0.98 (0.76-1.25) 0.850	0.98 (0.89-1.09) 0.707
PM_{2.5}	0.88 (0.57-1.35) 0.547	1.03 (0.84-1.27) 0.764	0.87 (0.57-1.35) 0.543	1.03 (0.84-1.27) 0.791	0.93 (0.63-1.38) 0.728	1.00 (0.81-1.23) 0.986
PM₁₀	1.04 (0.71-1.52) 0.852	0.98 (0.82-1.18) 0.863	1.03 (0.71-1.51) 0.874	0.98 (0.81-1.18) 0.811	1.08 (0.75-1.56) 0.688	0.95 (0.79-1.13) 0.542
PM_{coarse}	0.92 (0.66-1.30) 0.646	1.02 (0.88-1.17) 0.837	0.92 (0.65-1.28) 0.610	1.01 (0.87-1.17) 0.923	1.03 (0.75-1.42) 0.851	1.00 (0.87-1.15) 0.973
PM_{2.5abs}	1.16 (0.76-1.78) 0.485	0.97 (0.81-1.16) 0.749	1.15 (0.75-1.76) 0.520	0.96 (0.80-1.16) 0.687	1.18 (0.80-1.76) 0.404	0.93 (0.78-1.11) 0.428
MCP-1						
NO₂	0.61 (0.30-1.22) 0.162	0.81 (0.61-1.06) 0.129	0.61 (0.30-1.22) 0.162	0.81 (0.61-1.07) 0.131	0.54 (0.27-1.08) 0.081	0.81 (0.61-1.07) 0.135
NO_x	0.68 (0.40-1.17) 0.165	0.83 (0.67-1.04) 0.105	0.68 (0.40-1.17) 0.166	0.83 (0.67-1.04) 0.107	0.62 (0.36-1.07) 0.085	0.83 (0.67-1.04) 0.107
PM_{2.5mass}	0.50 (0.20-1.23) 0.130	0.91 (0.59-1.41) 0.684	0.50 (0.20-1.23) 0.130	0.91 (0.59-1.41) 0.686	0.48 (0.20-1.14) 0.096	0.97 (0.61-1.54) 0.903
PM₁₀	0.93 (0.41-2.08) 0.860	0.94 (0.64-1.39) 0.758	0.93 (0.41-2.09) 0.861	0.94 (0.64-1.39) 0.761	0.81 (0.36-1.86) 0.625	0.94 (0.63-1.41) 0.767
PM_{coarse}	0.66 (0.33-1.35) 0.258	0.98 (0.73-1.34) 0.923	0.66 (0.33-1.35) 0.258	0.99 (0.72-1.34) 0.924	0.54 (0.27-1.10) 0.088	0.99 (0.73-1.34) 0.931
PM_{2.5abs}	0.70 (0.28-1.71) 0.433	0.97 (0.66-1.43) 0.881	0.70 (0.28-1.72) 0.434	0.97 (0.66-1.43) 0.882	0.58 (0.24-1.41) 0.230	0.96 (0.64-1.42) 0.831

TNF-alpha						
NO₂	2.01 (1.19-3.40) 0.009	0.93 (0.76-1.15) 0.517	1.99 (1.18-3.35) 0.010	0.94 (0.76-1.16) 0.576	2.26 (1.31-3.88) 0.003	0.92 (0.74-1.15) 0.472
NO_x	1.74 (1.16-2.62) 0.008	0.93 (0.79-1.10) 0.417	1.73 (1.15-2.59) 0.009	0.94 (0.80-1.11) 0.473	1.91 (1.25-2.91) 0.003	0.93 (0.78-1.10) 0.387
PM_{2.5mass}	1.99 (1.01-3.93) 0.048	0.77 (0.56-1.07) 0.126	1.99 (1.01-3.92) 0.046	0.78 (0.56-1.08) 0.135	2.12 (1.07-4.20) 0.032	0.77 (0.54-1.11) 0.161
PM₁₀	1.24 (0.67-2.29) 0.499	1.08 (0.80-1.46) 0.594	1.25 (0.68-2.31) 0.474	1.10 (0.82-1.48) 0.535	1.41 (0.73-2.73) 0.311	1.12 (0.81-1.54) 0.499
PM_{coarse}	1.20 (0.69-2.07) 0.521	1.03 (0.82-1.31) 0.781	1.22 (0.71-2.10) 0.477	1.05 (0.83-1.33) 0.674	1.31 (0.74-2.33) 0.350	1.09 (0.85-1.40) 0.501
PM_{2.5abs}	1.12 (0.56-2.24) 0.742	1.09 (0.81-1.46) 0.574	1.15 (0.58-2.29) 0.684	1.11 (0.82-1.49) 0.499	1.23 (0.60-2.54) 0.566	1.14 (0.83-1.57) 0.417
IFN-gamma						
NO₂	1.03 (0.58-1.84) 0.917	0.95 (0.75-1.19) 0.652	1.03 (0.58-1.83) 0.927	0.95 (0.75-1.20) 0.671	0.98 (0.54-1.79) 0.955	0.93 (0.73-1.19) 0.564
NO_x	1.01 (0.64-1.59) 0.968	0.95 (0.79-1.14) 0.567	1.01 (0.64-1.58) 0.979	0.95 (0.79-1.14) 0.587	0.97 (0.60-1.55) 0.893	0.93 (0.77-1.13) 0.474
PM_{2.5mass}	1.11 (0.52-2.34) 0.791	0.93 (0.65-1.33) 0.677	1.11 (0.52-2.34) 0.790	0.93 (0.65-1.33) 0.686	1.01 (0.48-2.14) 0.976	0.86 (0.58-1.28) 0.451
PM₁₀	0.96 (0.50-1.85) 0.907	0.87 (0.64-1.20) 0.401	0.96 (0.50-1.86) 0.913	0.88 (0.64-1.20) 0.412	0.85 (0.42-1.71) 0.651	0.81 (0.58-1.14) 0.233
PM_{coarse}	1.28 (0.72-2.29) 0.397	0.93 (0.73-1.20) 0.576	1.29 (0.72-2.31) 0.388	0.94 (0.73-1.20) 0.604	1.15 (0.63-2.10) 0.654	0.89 (0.68-1.15) 0.362
PM_{2.5abs}	1.19 (0.57-2.48) 0.640	0.91 (0.66-1.24) 0.539	1.20 (0.58-2.50) 0.627	0.91 (0.66-1.25) 0.559	1.09 (0.51-2.33) 0.825	0.86 (0.61-1.20) 0.373

¹ Calculated based upon the estimate of the main effect and the multiplicative interaction term in model 2

² Adjusted for sex

³ Adjusted for sex, parental education, siblings, current environmental tobacco smoke at child's home, visible signs of mold or dampness in the home, body mass index

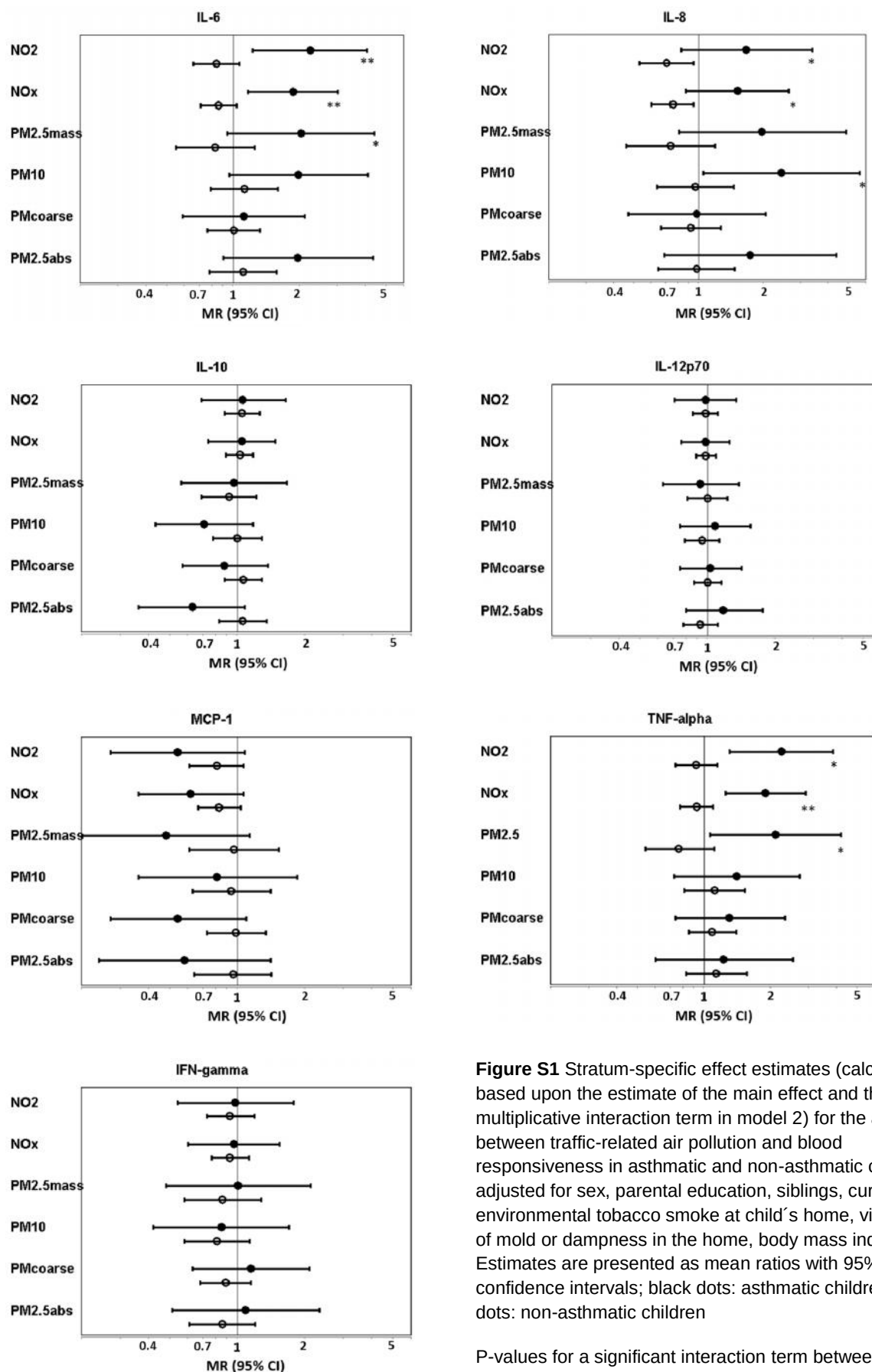


Figure S1 Stratum-specific effect estimates (calculated based upon the estimate of the main effect and the multiplicative interaction term in model 2) for the association between traffic-related air pollution and blood responsiveness in asthmatic and non-asthmatic children adjusted for sex, parental education, siblings, current environmental tobacco smoke at child's home, visible signs of mold or dampness in the home, body mass index (N=81). Estimates are presented as mean ratios with 95% confidence intervals; black dots: asthmatic children; white dots: non-asthmatic children

P-values for a significant interaction term between asthma status and exposure are indicated by asterisks (* p-value <0.05; ** p-value <0.01).

Table S4 Stratum specific effect estimates¹ (MR: Means ratio) of traffic-related air pollution exposure on whole blood cytokine responsiveness in asthmatic and non-asthmatic children – results based on models with interaction term

	Model 2sens1² (N=73)		Model 2sens2³ (N=83)		Model 2sens3⁴ (N=70)	
	Asthma +	Asthma-	Asthma +	Asthma-	Asthma +	Asthma-
	MR (95% CI) p-value	MR (95% CI) p-value	MR (95% CI) p-value	MR (95% CI) p-value	MR (95% CI) p-value	MR (95% CI) p-value
IL-6						
NO₂	3.25 (0.67-15.78) 0.144	0.88 (0.69-1.13) 0.309	2.32 (1.22-4.41) 0.011	0.87 (0.68-1.10) 0.249	2.21 (1.01-4.82) 0.047	0.98 (0.70-1.36) 0.889
NO_x	2.53 (0.73-8.71) 0.142	0.89 (0.73-1.08) 0.245	1.90 (1.15-3.13) 0.012	0.88 (0.73-1.06) 0.184	1.84 (0.99-3.39) 0.052	0.97 (0.74-1.26) 0.803
PM_{2.5mass}	2.74 (0.84-8.88) 0.093	0.86 (0.58-1.26) 0.437	2.02 (0.83-4.91) 0.122	0.84 (0.57-1.24) 0.393	2.18 (0.65-7.31) 0.205	0.98 (0.63-1.54) 0.933
PM₁₀	1.83 (0.58-5.83) 0.305	1.18 (0.84-1.67) 0.334	2.11 (1.02-4.37) 0.045	1.18 (0.84-1.65) 0.338	2.25 (0.89-5.73) 0.088	1.29 (0.90-1.85) 0.169
PM_{coarse}	1.87 (0.74-4.73) 0.186	1.02 (0.78-1.34) 0.880	1.18 (0.60-2.34) 0.628	1.01 (0.77-1.33) 0.937	1.61 (0.51-5.06) 0.414	1.16 (0.85-1.59) 0.359
PM_{2.5abs}	2.45 (0.62-9.71) 0.204	1.17 (0.83-1.64) 0.371	2.37 (1.03-5.43) 0.042	1.17 (0.84-1.63) 0.359	2.83 (1.00-8.01) 0.051	1.29 (0.87-1.90) 0.200
IL-8						
NO₂	3.47 (0.63-19.04) 0.151	0.74 (0.57-0.97) 0.030	1.71 (0.82-3.59) 0.153	0.75 (0.57-0.99) 0.043	1.53 (0.65-3.59) 0.327	0.83 (0.58-1.19) 0.306
NO_x	2.61 (0.69-9.88) 0.159	0.79 (0.63-0.97) 0.026	1.52 (0.86-2.70) 0.152	0.79 (0.64-0.99) 0.038	1.41 (0.72-2.75) 0.314	0.85 (0.64-1.14) 0.275
PM_{2.5mass}	4.40 (1.25-15.54) 0.021	0.73 (0.48-1.10) 0.131	2.51 (0.92-6.85) 0.072	0.77 (0.50-1.19) 0.234	1.98 (0.54-7.31) 0.304	0.93 (0.57-1.51) 0.775
PM₁₀	2.95 (0.83-10.49) 0.094	0.95 (0.66-1.39) 0.810	2.44 (1.06-5.62) 0.035	0.99 (0.67-1.45) 0.945	2.87 (1.05-7.86) 0.040	1.11 (0.75-1.64) 0.600
PM_{coarse}	2.19 (0.79-6.06) 0.130	0.91 (0.67-1.22) 0.515	1.11 (0.51-2.41) 0.792	0.91 (0.67-1.25) 0.573	1.15 (0.33-3.99) 0.827	1.07 (0.76-1.51) 0.704
PM_{2.5abs}	3.11 (0.68-14.25) 0.144	0.97 (0.67-1.41) 0.864	1.98 (0.76-5.18) 0.162	0.99 (0.67-1.46) 0.969	2.18 (0.69-6.87) 0.184	1.09 (0.71-1.68) 0.684

TNF-alpha						
NO₂	4.82 (1.21-19.24) 0.026	0.94 (0.76-1.17) 0.577	2.34 (1.34-4.08) 0.003	0.95 (0.77-1.17) 0.632	2.00 (1.05-3.81) 0.035	1.01 (0.77-1.32) 0.955
NO_x	3.45 (1.17-10.21) 0.025	0.94 (0.79-1.12) 0.479	1.94 (1.25-2.99) 0.003	0.95 (0.81-1.12) 0.549	1.74 (1.05-2.89) 0.032	1.00 (0.80-1.24) 0.977
PM_{2.5mass}	3.62 (1.32-9.97) 0.013	0.78 (0.56-1.08) 0.136	2.51 (1.18-5.37) 0.018	0.81 (0.58-1.13) 0.210	2.17 (0.81-5.85) 0.126	0.86 (0.59-1.24) 0.419
PM₁₀	1.54 (0.55-4.32) 0.416	1.09 (0.80-1.49) 0.574	1.33 (0.69-2.55) 0.393	1.12 (0.83-1.51) 0.449	1.36 (0.62-2.99) 0.447	1.17 (0.86-1.59) 0.314
PM_{coarse}	2.42 (1.08-5.44) 0.032	1.05 (0.83-1.33) 0.702	1.26 (0.69-2.28) 0.451	1.06 (0.84-1.35) 0.609	1.98 (0.77-5.07) 0.155	1.12 (0.86-1.45) 0.403
PM_{2.5abs}	1.61 (0.47-5.53) 0.453	1.10 (0.81-1.49) 0.543	1.26 (0.60-2.64) 0.545	1.12 (0.83-1.51) 0.464	1.26 (0.52-3.07) 0.610	1.15 (0.82-1.60) 0.414

Calculated based upon the estimate of the main effect and the multiplicative interaction term in model 2

Adjusted for sex; children with anti-inflammatory asthma medication use have been excluded

³ Adjusted for sex and parental allergy

⁴ Adjusted for sex; children with parental-reported doctor-diagnosis of eczema or hay-fever at the age of six have been excluded

Table S5 Stratum specific effect estimates¹ (MR: Means ratio) of traffic-related air pollution exposure on whole blood cytokine responsiveness in asthmatic and non-asthmatic children – results based on models with interaction term

	Model 2sens4 ² (N=85)		Model 2sens5 ³ (N=86)		Model sens6 ⁴ (N=86)	
	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value	Asthma + MR (95% CI) p-value	Asthma- MR (95% CI) p-value
IL-6						
NO₂	2.09 (1.17-3.76) 0.013	0.90 (0.71-1.14) 0.386	2.15 (1.19-3.88) 0.011	0.87 (0.69-1.10) 0.254	2.17 (1.20-3.90) 0.010	0.87 (0.69-1.10) 0.241
NO_x	1.77 (1.12-2.80) 0.014	0.91 (0.75-1.10) 0.312	1.81 (1.14-2.87) 0.012	0.88 (0.73-1.07) 0.194	1.82 (1.15-2.87) 0.011	0.88 (0.73-1.06) 0.181
PM_{2.5mass}	1.92 (0.89-4.14) 0.096	0.87 (0.59-1.27) 0.463	1.88 (0.86-4.12) 0.116	0.86 (0.59-1.25) 0.423	1.89 (0.87-4.12) 0.108	0.83 (0.57-1.22) 0.350
PM₁₀	1.81 (0.90-3.63) 0.094	1.19 (0.85-1.66) 0.313	2.03 (1.02-4.03) 0.043	1.18 (0.85-1.64) 0.319	2.05 (1.04-4.04) 0.038	1.17 (0.84-1.63) 0.349
PM_{coarse}	1.16 (0.63-2.14) 0.630	1.03 (0.79-1.35) 0.828	1.22 (0.66-2.27) 0.525	1.02 (0.78-1.33) 0.904	1.22 (0.66-2.26) 0.531	1.00 (0.77-1.32) 0.974
PM_{2.5abs}	1.93 (0.89-4.22) 0.098	1.18 (0.85-1.65) 0.319	2.21 (1.03-4.74) 0.041	1.17 (0.84-1.62) 0.355	2.26 (1.06-4.82) 0.036	1.16 (0.83-1.60) 0.387
IL-8						
NO₂	1.45 (0.73-2.88) 0.283	0.75 (0.57-0.99) 0.045	1.43 (0.73-2.82) 0.296	0.73 (0.55-0.95) 0.020	1.49 (0.76-2.92) 0.251	0.73 (0.55-0.95) 0.022
NO_x	1.35 (0.79-2.31) 0.269	0.79 (0.63-0.99) 0.039	1.33 (0.79-2.26) 0.288	0.77 (0.62-0.96) 0.018	1.37 (0.81-2.33) 0.242	0.77 (0.62-0.96) 0.018
PM_{2.5mass}	1.89 (0.78-4.60) 0.159	0.73 (0.47-1.13) 0.163	1.77 (0.73-4.32) 0.210	0.73 (0.48-1.12) 0.149	1.88 (0.78-4.54) 0.162	0.68 (0.44-1.06) 0.086
PM₁₀	1.91 (0.85-4.31) 0.117	0.96 (0.65-1.41) 0.822	1.95 (0.88-4.30) 0.098	0.95 (0.65-1.39) 0.798	2.10 (0.96-4.60) 0.063	0.93 (0.63-1.36) 0.698
PM_{coarse}	1.00 (0.49-2.03) 0.998	0.91 (0.66-1.24) 0.539	1.05 (0.52-2.12) 0.891	0.90 (0.66-1.22) 0.493	1.04 (0.51-2.10) 0.916	0.87 (0.64-1.19) 0.383
PM_{2.5abs}	1.47 (0.59-3.70) 0.409	0.97 (0.66-1.44) 0.892	1.58 (0.65-3.83) 0.312	0.97 (0.66-1.41) 0.858	1.68 (0.69-4.09) 0.249	0.94 (0.64-1.38) 0.760

TNF-alpha						
NO₂	2.02 (1.20-3.38) 0.008	0.96 (0.78-1.19) 0.716	1.98 (1.17-3.34) 0.010	0.94 (0.76-1.16) 0.562	1.99 (1.18-3.35) 0.009	0.93 (0.76-1.15) 0.528
NO_x	1.74 (1.16-2.61) 0.007	0.95 (0.81-1.13) 0.588	1.72 (1.14-2.59) 0.010	0.94 (0.80-1.11) 0.463	1.73 (1.15-2.59) 0.009	0.93 (0.79-1.10) 0.426
PM_{2.5mass}	1.99 (1.01-3.90) 0.046	0.82 (0.59-1.14) 0.235	1.99 (1.00-3.93) 0.049	0.78 (0.56-1.08) 0.136	2.00 (1.02-3.91) 0.044	0.75 (0.54-1.04) 0.089
PM₁₀	1.31 (0.70-2.44) 0.400	1.16 (0.86-1.57) 0.320	1.23 (0.66-2.29) 0.508	1.10 (0.82-1.48) 0.531	1.26 (0.68-2.33) 0.456	1.09 (0.81-1.46) 0.589
PM_{coarse}	1.22 (0.71-2.10) 0.461	1.10 (0.87-1.40) 0.432	1.22 (0.71-2.11) 0.467	1.05 (0.83-1.33) 0.663	1.22 (0.71-2.10) 0.472	1.04 (0.82-1.32) 0.754
PM_{2.5abs}	1.20 (0.59-2.42) 0.614	1.17 (0.87-1.57) 0.311	1.14 (0.57-2.27) 0.708	1.11 (0.83-1.49) 0.490	1.17 (0.59-2.32) 0.657	1.10 (0.82-1.47) 0.540

¹Calculated based upon the estimate of the main effect and the multiplicative interaction term in model 2
Adjusted for sex and differences in time between blood sampling and start of incubation

³Adjusted for sex and season of blood sampling

⁴Adjusted for sex and age of blood sampling

Table S6 Annual mean concentrations of air pollutants measured between 2002 and 2009 at the local governmental monitoring station Wesel Feldmark

Year	PM ₁₀		NO ₂		NO _x	
	N*	Mean [µg/m ³]	N*	Mean [µg/m ³]	N*	Mean [µg/m ³]
2002	338	23.94	344	32.67	344	22.59
2003	357	21.72	325	37.98	325	26.42
2004	361	23.40	347	37.31	347	24.89
2005	357	23.26	353	34.03	353	25.17
2006	364	23.88	352	35.74	352	26.71
2007	358	26.58	291	33.60	291	24.37
2008	359	21.47	314	33.26	314	24.95
2009	354	24.17	325	35.98	325	26.64

*Number of measurements

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