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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 LISAplus 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 chemotactic 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 (NO_2), nitrogen oxides (NOx), particulate matter with an aerodynamic diameter $<10\,\mu m$ (PM_{10}), particulate matter with an aerodynamic diameter $<2.5\,\mu m$ (PM2.5mass), 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 NO_2 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 $\mu g/m^3$ NO_2. The interaction term between asthma status and NO_2 exposure was significant. Results for NO_x, PM_{10}, $PM_{2.5mass}$ 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.

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-Fahrländer 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 μm (PM_{10}), particulate matter with an aerodynamic diameter < 2.5 μm ($PM_{2.5mass}$), coarse particulate matter (PM_{coarse}) and $PM_{2.5absorbance}$ ($PM_{2.5abs}$) 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 $PM_{2.5mass}$, 69% and 63% for PM_{10}, 97% and 95% for $PM_{2.5abs}$, 66% and 57% for PM_{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 μg/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 chemotactic protein-1 (MCP-1), tumour necrosis factor- alpha (TNF-alpha) and interferon-gamme (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