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Intrauterine exposure to lead may enhance sensitization to common inhalant allergens in early childhood: A prospective prebirth cohort study

Wieslaw Jedrychowski [a,*], Frederica Perera [b], Umberto Maugeri [c], Rachel L. Miller [b,d], Maria Rembiasz [e], Elzbieta Flak [a], Elzbieta Mroz [a], Renata Majewska [a], Marek Zembala [f]

[a] Epidemiology and Preventive Medicine, Jagiellonian University Medical College, 7 Kopernika Str., Krakow, Poland
[b] Center for Children's Environmental Health, Mailman School Public Health, Columbia University, New York, NY, USA
[c] Institute for Clinical Medicine and Rehabilitation, Pavia, Italy
[d] Division of Pulmonary, Allergy and Critical Care Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA
[e] Municipal Children's Hospital, Krakow, Poland
[f] Department of Clinical Immunology, the Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland

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ABSTRACT

Background: Several in vivo and in vitro studies have shown that metal-rich particles may enhance allergic responses to house dust mites and induce an increased release of allergy-related cytokines.
Objectives: The main goal of this analysis is to define the possible association of intrauterine exposure to lead and mercury with the occurrence of skin sensitization to common aeroallergens in early childhood.
Material and methods: The present study refers to a sample of 224 women in the second trimester of pregnancy recruited from Krakow inner city area who had full term pregnancies and whose children underwent skin prick testing (SPT) at the age of 5. Lead and mercury levels were assessed in cord blood and retested in children at age of 5 years. Aeroallergen concentrations in house dust were measured at the age of 3 years. The main health outcome (atopic status) was defined as the positive SPT to at least one common aeroallergen (Der f1, Der p1, Can f1 and Fel d1) at the age of 5 years. In the statistical analysis of the association between atopic status of children and exposure to metals, the study considered a set of covariates such as maternal characteristics (age, education, atopy), child's gender, number of older siblings, prenatal (measured via cord blood cotinine) and postnatal environmental tobacco smoke together with exposure to polycyclic aromatic hydrocarbons (PAH) as measured by PAH-DNA adducts.
Results and conclusion: In the binary regression analysis, which controlled for the confounders, the risk ratio (RR) estimate for atopic sensitization was significantly associated with the lead exposure (RR=2.25, 95%CI: 1.21−4.19). In conclusion, the data suggest that even very low-level of prenatal lead exposure may be implicated in enhancing sensitization to common aeroallergens in early childhood.

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1. Introduction

Sensitization to aeroallergens is very common in childhood and is often one of the first manifestations of asthma and allergic rhinitis. Allergic diseases are often evident within the first months of life, which highlights the need to investigate the role of prenatal and very early life events possibly leading to the onset of atopy and allergic march. There are many environmental conditions responsible for atopy to common aeroallergens, including indoor/outdoor allergens and pollutants generated by traffic (Schwarze et al., 2006; Ruszniak et al., 1998; Devalia et al., 1996). Identifying the specific causative agents contained in traffic-related pollution is one of many challenges because of its multiple constituents. After combustion of diesel fuel, the exhaust components aggregate into discrete spherical respirable particles approximately 0.1–0.5 μm in diameter, consisting of an elemental carbon core with a large surface area to which polycyclic aromatic hydrocarbons (PAHs), chemicals and transition metals attach.

Epidemiologic observations on urban, industrial pollution across eastern Germany strongly suggest that the exposure to metal-rich pollutants (zinc, magnesium, lead, copper, and cadmium) may largely account for regional differences in prevalence of allergic sensitization in children (Heinrich et al., 1999). The differences correlate with several-fold higher levels of these metal presences in fine particulate matter (Gavett et al., 2003). However,

neither multi-pollutant modeling nor the adjustment of individual effects of lead after controlling for other metals was carried out in the study.

The hypothesis of Rabinowitz et al. (1990) that early exposure to lead is a risk factor for childhood asthma has been under consideration for about two decades. The hypothesis was supported by observations that both in atopic and non-atopic asthmatics lead have been associated with the increased production of total immunoglobulin E (IgE) (Beeh et al., 2000; Romanet-Manent et al., 2002). Furthermore, other studies reported an association between lead and increased IgE in young children (Lutz et al., 1999; Sun et al., 2003) and an elevated risk of asthma among children exposed to lead was observed in certain population subgroups (Joseph et al., 2005) although the results were not always statistically significant.

Although exposure to low or moderate lead levels does not produce a marked loss of immune cells, subtle lead-related changes in the immune cell population can be functionally harmful. A strong feature of lead-induced immunotoxicity is a pronounced shift in the balance in helper T cell function, from T helper type 1 (Th1) toward T helper type 2 (Th2) responses at the expense of functions (Dieteret et al., 2004). This may alter the character and array of immune responses and subsequently influence host susceptibility to various diseases (Gupta et al., 2002; McCabe et al., 2001). Until now, human studies on the sensitization effects of intrauterine lead exposure have been very sparse. We hypothesize that intrauterine exposure to transition metals (lead, mercury) may influence the sensitization to common aeroallergens in early childhood.

2. Material and methods

The subjects included in this analysis were 323 women recruited from January 2001 to February 2004 in Krakow inner city area, who gave birth to term babies and completed the 5-year follow-up. Out of 323 children who completed the 5-year follow-up, 254 underwent SPT at the age of 5 years.

Women attending ambulatory prenatal clinics in the first and second trimesters of pregnancy were eligible for the study. The enrollment included only non-smoking women with singleton pregnancies ages 18–35, who were free from chronic diseases such as diabetes or hypertension. Prior to participation, women read and signed an informed consent. The Jagiellonian University Bioethical Committee approved the research.

The detailed description of the study design was presented elsewhere (Jedrychowski et al., 2003). Upon enrollment, a detailed questionnaire was administered to each subject to elicit information on demographic data, house characteristics, date of the last menstrual period (LMP), medical and reproductive history, occupational hazards, alcohol consumption, nutritional habits, and smoking practices of others present in the home. Maternal atopy was defined as reported medical diagnosis of eczema, asthma or hay fever. Gestational age at birth denotes the interval between the last day of the mother's LMP and the date of birth. Prenatal environmental tobacco smoke (ETS) was defined by the cord blood cotinine levels above median and postnatal ETS was assumed if at least one of the household members was an active smoker over the follow-up period.

Prenatal burden of exposure to heavy metals was assessed at delivery (cord blood levels of lead and mercury) and the measurements were repeated in children at 5 years of age. Aeroallergen concentrations in house dust was measured at 3 years of age. Atopic status of children was defined as the sensitization to at least one common aeroallergen (Der f1, Der p1, Can f1 and Fel d1) measured by the skin prick testing (SPT) at the age of 5 years. In the statistical analysis of the association between atopic status of children and prenatal exposure to environmental hazards, the study considered a set of potential confounders including maternal characteristics (age, education, atopy), child's gender, parity, environmental tobacco smoke, the total concentration of aeroallergens in the house dust and prenatal exposure to PAHs. The analysis used PAH-DNA adducts as they are estimated to reflect the absorbed cumulative dose of PAHs exposure by the fetus during the prenatal period.

2.1. Dosimetry of heavy metals in blood

A cord blood sample (30–35 ml) and the identical amount of maternal blood at delivery was drawn into two separate vacutainer tubes treated with ethylene diamine tetra-acetate (EDTA). The tubes were inverted several times to mix the EDTA and the blood to prevent coagulation. Within 8 h of blood collection, the blood

samples were transported to the clinical biochemistry laboratory at the University Hospital in Krakow for processing and storage. Packed red blood cells and plasma samples were separated and stored in liquid nitrogen in the laboratory prior to shipment to Columbia University. From Columbia University, the samples were sent to the Centers for Disease Control and Prevention (CDC) for chemical analysis. Blood samples for lead analysis were refrigerated without any processing. Whole blood lead concentrations were determined using inductively coupled plasma mass spectrometry CLIA'88 method "Blood lead cadmium mercury ICPMS_ITB001A". This multi-element analytical technique is based on quadruple ICP-MS technology (CDC Centers for Disease Control and Prevention, 2003). Mercury levels were measured at the CDC by Zeeman graphite furnace atomic absorption spectrometry, using a phosphate/Triton X-100/nitric acid matrix modifier. Cold vapor atomic spectrometry following chemical reduction of mercury compounds was used to measure total mercury in whole blood. Identical analytical methods were used for measurements of metals in the capillary blood.

2.2. Dosimetry of PAH-DNA adducts

The buffy coat, packed red blood cells, and plasma were separated and stored at $-70\,^{\circ}$C. BaP-DNA adducts in extracted white blood cell (WBC) DNA were analyzed using the high-performance liquid chromatography (HPLC) fluorescence method of Alexandrov et al. (1992), which detects BaP tetraols. The assay gives zero values when unexposed calf thymus DNA is tested (D. Tang, personal communication). The method has a variation coefficient of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides. HPLC analysis of DNA samples for BaP-DNA adducts was performed in batches, with 18-paired maternal and newborn samples in the same batch.

2.3. Dosimetry of cord blood cotinine

The serum cotinine concentration was measured at CDC using the sensitive isotope-dilution high-performance liquid chromatographic/atmospheric pressure ionization tandem spectrometric (LC/MS/MS) procedure (Bernert et al., 1997, 2000). The limit of detection (LOD) was < 0.050 ng/mL and about 25% of specimens had cotinine levels below the LOD. Maternal blood cotinine level below 15.0 ng/L was considered the borderline value separating smokers from non-smokers (Jarvis et al., 1987; Peacock et al., 1998).

2.4. Dosimetry of house dust allergens

When the children reached age 3, house dust samples were collected from kitchen floors and from children's bedrooms and the mattresses. Floors were sampled over a 2-min period, from a 2 m $\times$ 2 m frame; in bedrooms, samples were collected adjacent to the bed, and in the kitchen where the child used to spend time. Parents were requested not to clean the mattresses, sweep or vacuum these floors for 48 h prior to sampling. The same vacuum cleaner was used to collect dust samples from all household sites, and trained staff performed the dust collection. To avoid cross-contamination between samples from different sites, vacuum cleaner parts were cleaned with wet cloths and dried after each sampling. All dust samples were sealed in plastic bags and sent to the laboratory of the Department of Clinical Immunology at the Polish-American Institute of Pediatrics (Jagiellonian University Medical College), where they were stored at 4 °C, under desiccant, until they were extracted. Extracted dust samples were assayed for Der f1, Der p1, Can f1 and Fel d1 by ELISA (Indoor Biotechnologies, Chester, United Kingdom). House dust cumulative exposure (dichotomized by median concentrations of allergens in the house) was used to define the exposure status of the household.

2.5. Ascertainment of atopic status

All 5-year olds who completed the follow-up underwent SPT for 4 common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, dog and cat hair). The results were read after 15 min by measuring the largest diameter of the wheal. Sensitization status was ascertained as a wheal-reaching diameter 3 mm and greater than the histamine control.

2.6. Statistical analysis

Statistical analysis was performed in order to assess a possible association between atopic status of children in 5-year olds and prenatal exposure to heavy metals. After removing the outliers of cord blood lead concentrations, defined by the values above 95th percentile ($\geq 2.5\,\mu g/dL$), the final statistical analysis was comprised of 224 children. In the initial descriptive analysis, geometric means of continuous variables were used because of their skewed distribution; this was followed by the univariate analysis of the association between dependent variable and exposure variables. To assess the risk ratio (RR) for atopic status of children associated with a given exposure, a conventional binary outcome logistic regression model was used after accounting for a priori selected factors such as child's gender,

maternal age and education, maternal atopy, parity and ETS. We used multivariate fractional polynomials to test the linearity of the association of cord blood lead levels and other exposure variables. Due to cord blood lead level and other exposure variables that are continuous, their relationships with atopic status may not be linear. Multivariate fractional polynomial algorithm searches the powers of these variables to determine the best-fitting relationship with atopic status. The fitting procedure involved transforming continuous variables, using a class of eight possible functions to identify the one that provides the best fit (Royston and Sauerbrei, 2005; Hilbe, 2009). In all binary regression models main exposure variables were introduced in an untransformed form and covariates as categorical or indicator variables. In the first step, we separately calculated crude RR estimates for each main exposure variable. In the next step, the RR estimates were adjusted for maternal age and finally, the full model including all covariates was performed. All statistical analyses were performed with STATA 11.1 version software for Windows (Kohler and Kreuter, 2005; STATA Reference Manual, Release 11, 2009).

3. Results

Table 1 presents the overall characteristics of children in the study grouped by their atopic status. It demonstrates that there are no statistical differences in characteristics of atopic and non-atopic children. Children who did not have an SPT measurement did not differ from those who attended the testing; therefore, the study subsample is representative of the larger cohort.

The overall geometric mean of the cord blood lead was 1.16 µg/dL (95%CI: 1.12–1.22) and appeared to be significantly lower than that in maternal blood (1.60, 95%CI: 1.52–1.67). The cord blood lead level was significantly higher in atopic children (1.34 µg/dL, 95%CI: 1.23–1.47) compared to non-atopic (1.14 µg/dL, 95%CI: 1.09–1.19) (Table 2). The mean lead concentration in maternal blood was also significantly higher in atopic children (1.85 µg/dL, 95%CI: 1.68–2.04) than that in non-atopic children (1.55 µg/dL, 95%CI: 1.47–1.63). Mean blood lead level in 5-year olds was about twofold higher than that found in cord blood; however, no difference in blood lead levels across the atopy groups was observed.

In contrast, the overall mean cord blood mercury concentration was higher (0.88 µg/dL, 95%CI: 0.81–0.95) than in maternal blood (0.60 µg/dL 95%CI: 0.54–0.67) and there was an insignificant difference between cord blood and maternal blood levels between the groups of children with atopy and without it. Mean blood mercury level in 5-year olds was about twofold lower than that found in cord blood; however, no difference in blood mercury levels across the atopy groups was observed. The correlation coefficients between lead and mercury concentrations in cord and maternal blood were highly significant.

Geometric means for cotinine, lead, mercury and PAH adducts in cord blood and lead and mercury in 5-year-olds did not differ

Table 1
Characteristics of the study sample grouped by the presence of atopy* in 5-year olds.

Variables	Non-atopic $N=187$	Atopic $N=37$	Total $N=224$
Maternal age			
Mean	27.62	28.38	27.75
SD	3.34	3.47	3.37
Maternal education			
Elementary+medium n (%)	52 (27.8)	16 (43.2)	68 (30.4)
Higher n (%)	135 (72.2)	21 (56.8)	156 (69.6)
Maternal atopy (+)	45 (24.1)	10 (27.0)	55 (24.6)
Parity			
1 n (%)	121 (64.7)	22 (59.5)	143 (63.8)
≥ 2 n (%)	66 (35.3)	15 (40.5)	81 (36.2)
Gender			
Boys n (%)	91 (48.7)	19 (52.5)	110 (49.1)
Girls n (%)	96 (51.3)	18 (47.5)	114 (50.9)
Gestational age			
Mean (weeks) > 36	39.58	39.32	39.54
SD	1.092	1.107	1.096
Breastfeeding exclusive > 3 months n (%)	103 (55.1)	19 (51.4)	122 (54.5)

Table 2
Burden of prenatal and postnatal exposure to environmental hazards grouped by the atopic status of 5-year olds included in the analysis.

Variables	Non-atopic $N=187$	Atopic $N=37$	Total $N=224$
Prenatal ETS (+) n (%)	45 (24.1)	12 (32.4)	57 (25.4)
Postnatal ETS (+) 1–5 years n (%)	41 (22.8)	6 (17.1)	47 (21.9)
Missing data	7	2	9
Postnatal allergen in house n (%)	106 (79.1)	22 (81.5)	128 (79.5)
Missing data	53	10	63
Cord blood lead			
Geometric mean	1.14	1.34	1.16
(95%CI)	(1.09–1.19)	(1.23–1.47)	(0.12–1.22)
Maternal blood lead			
Geometric mean	1.55	1.85	1.60
(95%CI)	(1.47–1.63)	(1.68–2.04)	(1.52–1.67)
Missing data	37	6	43
Cord blood mercury			
Geometric mean	0.86	0.96	0.88
(95%CI)	(0.79–0.95)	(0.82–1.13)	(0.81–0.95)
Missing data	7	3	10
Maternal blood mercury			
Geometric mean	0.61	0.58	0.60
(95%CI)	(0.54–0.61)	(0.45–0.73)	(0.54–0.67)
Missing data	46	9	55
Cord blood cotinine			
Geometric mean	0.12	0.09	0.11
(95%CI)	(0.09–0.15)	(0.07–0.13)	(0.09–0.14)
Missing date	5	1	6
Cord blood PAH adducts			
Geometric mean	0.23	0.21	0.22
(95%CI)	(0.21–0.24)	(0.18–0.25)	(0.21–0.24)
Missing data	13	5	18
Postnatal blood lead			
Geometric mean	2.02	2.11	2.04
(95%CI)	(1.93–2.12)	(1.91–2.33)	(1.95–2.12)
Missing data	14	4	18
Postnatal blood mercury			
Geometric mean	0.44	0.49	0.45
(95%CI)	(0.40–0.49)	(0.39–0.61)	(0.41–0.49)
Missing data	14	4	18

between atopic and non-atopic children. While there was a significant correlation between lead levels measured in cord blood at age of 5 years (rho=0.289, 95%CI: 0.162–0.407), the correlation between mercury measurements in cord blood and those in 5-year olds was insignificant (rho=0.115, 95%CI: −0.022–0.248).

Among children who underwent SPT for 4 common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, dog and cat hair), 37 children (16.5%, 95%CI: 11.6–21.4) developed a positive skin test to at least one inhalant allergen, and dust mites were the most common sensitizer (12.5%, 95%CI: 8.4–16.9). Only 5.8% (95%CI: 3.0–8.9%) and 3.1% (95%CI: 0.8–5.4%) of children were sensitized to cat and dog allergens, respectively. While 6.3% (95%CI: 3.50–10.3%) of children were sensitized to only one allergen, 6.7% (95CI: 3.8–10.8%) were sensitized to two and 3.5% (95%CI: 1.6–6.9%) to three allergens.

The frequency of atopy was significantly associated both with the cord blood lead (nonparametric trend $z=3.30$, $p=0.001$) and maternal blood lead level ($z_{trend}=2.74$. $p=0.006$). The corresponding trend estimates were for cord blood mercury ($z_{trend}=0.86$, $p=0.387$), cotinine ($z_{trend}=0.67$, $p=0.502$) and PAH-adducts ($z_{trend}=-0.98$, $p=0.326$). The frequency of atopy was associated neither with lead ($z_{trend}=0.80$, $p=0.425$) nor mercury levels ($z_{trend}=0.98$, $p=0.826$) measured in the blood of 5-year olds. The risk of atopy was associated neither with the mean level of HDM (Der f1+Der p1) nor with pet allergens measured in the third year of the follow-up (not shown here).

Table 3
Risk ratios for atopic status in 5-year olds related to environmental hazards measured in perinatal and postnatal periods. RR estimates derived from the binary regression models.

Exposure variables	Crude RR (95%CI)	RR[a] (95%CI)	RR[b] (95%CI)
Cotinine in cord blood	0.68 (0.22–2.08)	0.74 (0.29–3.00)	0.58 (0.16–2.10)
Lead in cord blood	2.25 (1.21–4.19)	2.20 (1.17–4.16)	2.28 (1.12–4.62)
Lead in maternal blood	1.95 (1.20–3.18)	1.81 (1.10–3.00)	1.72 (0.98–3.00)
Mercury in cord blood	1.06 0.66–1.71	1.00 (0.61–1.63)	1.11 (0.68–1.80)
Mercury in maternal blood	0.77 (0.37–1.59)	0.72 (0.34–1.51)	0.64 (0.28–1.45)
PAH adducts in cord blood	0.21 (0.13–3.52)	0.18 (0.02–2.92)	0.12 (0.01–1.85)
Blood lead in 5-year olds	1.12 (0.73–1.72)	1.16 (0.77–1.76)	1.10 (0.72–1.64)
Blood mercury in 5-year olds	1.43 (0.58–3.56)	1.32 (0.54–3.27)	1.76 (0.75–4.14)

[a] RR estimates adjusted only for maternal age (dichotomized by median).
[b] RR adjusted for child's, gender, parity, maternal age, maternal education, maternal atopy, and ETS variables.
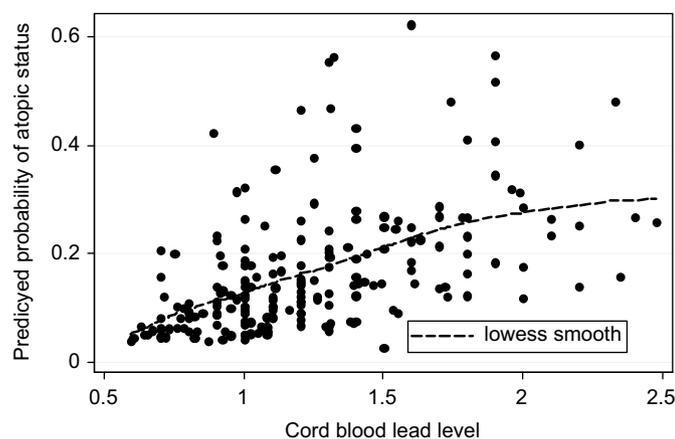

Fig. 1. Scatter plot and lowess line for predicted probability of atopic status in 5-year olds related to cord blood level (µg/dL) adjusted for covariates.

The unadjusted RR estimate for atopic status was significantly associated with cord blood lead concentrations (RR=2.25, 95%CI: 1.21–4.19). In the multivariable logistic regression models the RR estimate controlling for the effects of all covariates remained virtually the same (RR=2.28, 95%CI: 1.12–4.62) (Table 3). The inclusion of breastfeeding and house dust cumulative exposure (dichotomized by median concentrations of allergens in the house) did not affect the results. Fig. 1 presents the predicted probability of atopic status in 5-year olds related to cord blood lead concentrations after adjustment for covariates. Although atopy was significantly associated with maternal blood lead level measured at delivery, RR adjusted for all covariates (full regression model) appeared to be at borderline significance.

4. Discussion

The results of this study suggest that the sensitization to common aeroallergens in early childhood may be significantly enhanced by prenatal exposure to lead. This effect was not associated with other intrauterine exposures (mercury, cotinine or PAH-adducts) or with postnatal lead exposure of children. Although the effect of fetal lead exposure on the sensitization to common aeroallergens was adjusted for many potential confounders, it is possible that the intrauterine lead exposure may be a marker of other characteristics of children or an interaction between other exposures possibly involved in allergic sensitization, which were not taken into account in the present study.

The findings of our study agree with the previously mentioned epidemiologic observations between East and West Germany (Heinrich et al., 1999). Controlling for medical, socio-demographic,

and indoor factors, the authors found that children residing in a polluted county (containing heavy metal dust emissions from the smelters) had about a 50% increased lifetime prevalence of physician-diagnosed allergies, eczema, and bronchitis compared to children from the "clean county"; they also had about twice the number of respiratory symptoms such as wheeze, shortness of breath, and cough without cold. Sensitization to common aeroallergens measured by SPT was significantly higher in children from the polluted county than in those from the unpolluted area (OR=1.38; 95%CI: 1.02 – 1.86). Higher specific IgE levels were also more common in residents of the more polluted area (OR=1.75; 95%CI: 1.31–2.33). However, neither multi-pollutant modeling nor the adjustment of individual effects of lead, after controlling for other metals, was carried out in the study under discussion.

The present study is also consistent with in vivo and in vitro studies on the effects of metals on allergic sensitization. The hypothesis on the potential effect of transition metals on allergic diseases first came from the findings that metals induce IgE production (Prouvost-Danon et al., 1981; Murdoch et al., 1986). In fact, the main feature of lead-induced immunotoxicity is the shift of Th1-associated responses toward Th2-dependent cellular responses (Heo et al., 1996, 1997, 1998; McCabe and Lawrence, 1991; Lawrence and McCabe, 2002). Interestingly, in a Brown-Norway rat model of house dust mite allergy, intratracheal instillation of residual oil fly ash (ROFA), which is rich in metals such as zinc, lead, copper and cadmium, or its soluble metal constituents before HDM (house dust mite) sensitization increased serum specific IgE levels, immediate allergic responses, and exacerbated the inflammatory responses (Lambert et al., 1999, 2000, 2001; Gavett et al., 1999). Similar results have been seen in rats exposed to grass pollen and diesel exhaust particles (Steerenberg et al., 1999). Taken together, these studies suggest that exposure to transition metals may have influence on subsequent allergic responses.

The results from our study led us to believe that it is prenatal exposure rather than the postnatal blood lead levels that is more important in enhancing sensitization to common aeroallergens. This is in agreement with experimental studies on laboratory animals, which show that similar prolonged fetal/neonatal exposure to lead induces a comparable range of immune alterations as in the adult, however, at much lower exposure levels. Moreover, similar patterns of lead-induced immunotoxic alterations in terms of the dose–effect relationship have been observed in several animal species (Bunn et al., 2001a).

It is worthwhile to mention that exposure to lead during different periods of gestation can lead to different immune changes in postnatal period. At the comparable blood lead levels at birth, offsprings exposed to lead in early gestation had altered macrophage function but not the shift in Th1/Th2 balance; however, rats exposed in late gestation showed a clear shift toward Th2 that

could persist into adulthood (Bunn et al., 2001b). This would indicate that early-gestation-exposed fetuses may in later life preserve the pattern of allergic responses established in early gestation. This effect was also observed in chickens, where early embryonic exposures brought about postnatal macrophage alterations in the absence of Th1/Th2 effects, but late embryonic exposure was associated with lead-induced suppression of Th1 (Lee et al., 2001).

As in many previous epidemiologic studies, a major weakness of the present study is a relatively small study sample. Though retrospectively estimated power of our study (power=0.82, alpha=0.05) was sufficient to detect the significance of lead effects on the atopic status of children, the negative findings for other pollutants could be due to low statistical power. Additionally, our study sample may not be representative of all urban children in the country because enrollment covered children born to non-smoking mothers with singleton pregnancies between the ages of 18 and 35 years who were free from chronic diseases such as diabetes and hypertension. On the other hand, these inclusion criteria helped us eliminate from the study children who were at a greater risk for atopy due to maternal chronic diseases or active smoking. A positive element of the study was the relatively homogenous study sample in respect of socioeconomic status of the families and a small number of families, which changed their residences in the follow-up period. A strong feature of the study is the prospective cohort design and the statistical modeling of the association between atopic status of children and prenatal exposure considering a wide set of potential confounders.

In conclusion, our data suggest that even very low-levels of prenatal lead exposure may be implicated in enhancing sensitization to common aeroallergens in early childhood. We believe our results should be considered as an additional and important argument in the debate aimed at the need to revise environmental protection guidelines and set lower hygienic limits for lead exposure. Future studies should examine whether the effects observed here may be replicated using similar study design but with wider spectrum of prenatal indoor and outdoor environmental hazards, possibly testing interactions between other pollutants and considering other allergic symptoms.

Acknowledgments

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