

Exposure to air pollution in critical prenatal time windows and IgE levels in newborns

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The objective of this study was to analyze the mechanisms by which exposure to ambient air pollutants influences respiratory health may include altered prenatal immune development. To analyze associations between elevated cord serum Immunoglobulin E (IgE) levels and maternal air pollution exposure during each month of gestation. Total cord serum IgE was determined by the CAP system and mothers' total IgE levels by nephelometry for 459 births in the Czech Republic from May 1994 to mid-January 1997. Concentrations of polycyclic aromatic hydrocarbons (PAHs) and particulate matter < 2.5 microns in diameter (PM_{2.5}) were measured in ambient air, and arithmetic means were calculated for each gestational month. Log binomial regression models were used to estimate prevalence ratios (PR) for elevated cord serum IgE (≥ 0.9 IU/ml) adjusting for district of residence, year of birth, and in further models, for maternal IgE (a surrogate for atopy) and gestational season. Heterogeneity by maternal atopy status was evaluated for associations of air pollution and of cigarette smoke. In adjusted models, PAH and PM_{2.5} exposures in the second month of gestation were each associated with a lower prevalence of elevated cord serum IgE. For an average increase of 100 ng/m³ of PAHs, the PR was 0.69 (95% confidence interval (CI): 0.50, 0.95); for 25 µg/m³ increase in PM_{2.5}, the PR was 0.77 (95% CI: 0.55, 1.07). Conversely, exposures later in gestation were associated with a higher prevalence of elevated cord IgE: in the fifth month, the PR for PAH exposure was 1.64 (95% CI: 1.29, 2.08), while for PM_{2.5} in the sixth month, it was 1.66 (95% CI: 1.30, 2.13). In analyses stratified by maternal atopy, air pollutants were associated with altered cord serum IgE only among neonates with non-atopic mothers. Similarly, an association of cigarette smoke with elevated cord serum IgE was found only in non-atopic mothers. PAHs and PM_{2.5}, constituents of both ambient air pollution and cigarette smoke, appear to influence fetal immune development, particularly among infants whose mothers are not atopic.

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Epidemiological and experimental studies suggest that environmental pollutants such as automotive emissions (1) may play a role in increasing prevalence or severity of allergic disease (2). Environmental exposures may favor preferential induction of Immunoglobulin E (IgE) antibody responses, predisposing to allergic sensitization (3), or alternatively may act as adjuvants,

potentiating immune responses (2, 3). For example, extracts of polycyclic aromatic hydrocarbons (PAHs) from diesel exhaust particles enhance human IgE production in purified B cells (2). In addition, diesel particles can adsorb antigens onto their surfaces, acting as an adjuvant at the mucosal surface and enhancing IgE responses to common allergens (2).

The present report extends our prior findings that both acute and longer-term air pollution exposures are associated with alterations in lymphocyte immunophenotype distributions [decreased proportions of CD3⁺ CD4⁺ and CD8⁺ cells and increased B-lymphocyte (CD19⁺) fractions] (4, 5). Here, we report associations between air pollution exposures by gestational month and elevated levels of IgE in cord serum.

Methods

Study area and exposure assessment

An air quality field measurement program was begun in January 1992 by the Czech Ministry of Environment (5), which established two monitoring sites: one in Teplice district, located in Northern Bohemia, characterized by mining operations and numerous coal-fired power plants within an area of 469 km²; the other in Prachatic district in Southern Bohemia, with mainly light industry in an area of 1375 km². During the study period, the populations of Teplice and Prachatic Districts were about 120,000 and 50,000, respectively. The concentrations of particulate matter <2.5 microns in diameter (PM_{2.5}), PM₁₀, and PAHs in ambient air were measured daily in November through March, every third day in April through June and September through October, and every sixth day in July and August. Daily measurements of sulfur dioxide (SO₂) and total nitrogen oxides (NO_x), nitric oxide (NO) and nitrogen dioxide (NO₂), were conducted year-round.

Fine and coarse particle concentrations, as well as PAH concentrations, were determined from samples collected by a Versatile Air Pollution Sampler (VAPS, University Research Glass, Chapel Hill, NC, USA), as described previously (5). Concentrations of 12 PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene, indeno(cd)pyrene) were measured in ambient air and summed to obtain total 'PAH' levels for analysis.

For this investigation, we focused on monthly averages of air pollutants measured by this monitoring program. For days when PM_{2.5} and PAH concentrations were not measured (primarily in the summer season, when air pollution levels are relatively low and have low variability), imputation was conducted using daily measurements for SO₂ and NO_x, as described previously (5). Similar imputations were performed for individual PAHs missing for days when PAHs

were not measured, using all available pollutant values and taking conditional expectations derived by standard estimation procedures for a lognormal distribution (5).

Subject enrollment and data collection

Recruitment and data collection have been previously described (6). The 459 births analyzed in the present report occurred between May 1994 and mid-January 1997 and were part of the Pregnancy Outcome Study (POS). Nurses invited women delivering in Teplice and Prachatic to participate in the POS at the time of their hospital stay (6). About 90% of mothers who were approached agreed and after providing written informed consent completed self-administered questionnaires regarding reproductive histories, medical conditions and medications, smoking, alcohol and other lifestyle factors, and occupational information. Specially trained nurses reviewed these questionnaires for clarity and completeness.

A stratified random sample of about 20% of the POS mother/infant pairs was recruited into the Immune Biomarker Study. Preterm and low birth weight infants were oversampled relative to other births. Blood specimens were collected from the mother and the umbilical cord vein using venipuncture prior to delivery of the placenta to avoid contamination with maternal blood. Total IgE levels were measured in maternal and/or cord serum from 492 deliveries.

Data on the pregnancy, labor, delivery, and the neonate were abstracted from medical records, including birth weight, gestational age, and delivery date. We excluded 29 deliveries (one exceptionally long labor of >24 h and 28 with incomplete maternal sociodemographic data), leaving 463 with complete covariate information and maternal serum total IgE, of which 459 also had a cord serum total IgE measurement.

This study was approved by the Ethical Committee of the Regional Institute of Hygiene of Central Bohemia, Prague and the Human Subjects Committee of the University of California, Davis, School of Medicine.

Laboratory methods

Maternal peripheral venous blood sampled after the end of the second stage of labor and venous cord blood sampled immediately after labor were collected into heparinized vacutainers (10 ml, Vacuette®, Greiner), stored at +4°C in polystyrene boxes, and transported in coolers for analysis. Carrier Polymer System (CAP, Phar-

macia, Uppsala, Sweden) testing was used to analyze total IgE in cord serum. The detection limit was 0.35 IU/ml. Nephelometry [Behring Nephelometer 100 (BN 100), Behringwerke AG, Marburg, Germany] was used for the measurement of maternal IgE between 30 and 1000 IU/ml; for concentrations exceeding this range, the measurement was repeated with diluted serum. All measurements were taken in duplicate, and the mean values were used in the analyses. The coefficient of variation was 10% for cord IgE and 15% for maternal IgE levels.

Data handling and statistical methods

Medical record data and maternal questionnaire information were entered into electronic files in Prague at the Laboratory of Genetic Ecotoxicology, Academy of Sciences, Czech Republic. Outliers, implausible values, and inconsistencies across variables were identified and resolved in data cleaning. These data were linked with pollution and temperature data, and with cord and maternal serum IgE measurements.

Because, as expected, a large number (55%) of cord serum samples did not have detectable levels of IgE using the CAP method, we analyzed cord IgE categorically. We chose a biologically based cutpoint: the threshold of cord IgE considered indicative of risk for the development of atopic disease in later life, which is 0.9 IU/ml (7). We also conducted a separate analysis using the detection limit (0.35 IU/ml) as the cutpoint. Because both outcomes – detectable and elevated IgE levels – were common in the cord blood samples (45% and 29%, respectively), we fitted log binominal models. Thus, prevalence ratios (PRs) were obtained relating each set of air pollutants (PM_{2.5} and the summed PAHs) with the two dichotomized IgE outcomes.

Gestational months were calculated from the estimated date of conception based on the last menstrual period (LMP). Differences in reported LMP between mothers and clinicians, which can occur because of a variety of sources of variability and uncertainty (8), were resolved by the following algorithm: if the difference was <15 days, the mother's reported LMP was used (83.0% of 459 births). For differences between 335 and 395 days, the mother's estimate was adjusted by 365 days (1.3%), i.e., year is commonly off by one, when, for instance, the current rather than previous year is written down for the LMP. For differences between 15 and 334 days, the recorded birth weight was compared to standard Czech birth weight percentiles. If the mother's reported LMP yielded a gestational age

for which the birth weight was within the 5th to 95th percentiles from a standard population, it was used (6.3%). If not, the physician's estimate was used, provided the birth weight was within the 5th to 95th percentiles for that gestational age in a standard population (7.6%). If neither applied, the birth weight was used to impute LMP (1.7%).

We calculated PRs for cord serum IgE ≥ 0.9 IU/ml in relation to monthly average air pollution concentrations for each month of gestation. If a child was born preterm, then exposure for the last month of gestation was averaged for the number of completed days, e.g., air pollution exposure in the ninth month for a baby born at 8 months and 10 days was averaged across the last 9 days of exposure that ended on the day prior to birth.

Covariates of concern were identified from the literature and an empirical screen of the available variables. We incorporated these variables into a causal model represented by a directed acyclic graph (DAG) (9) to determine which variables should or should not be controlled to minimize confounding. The DAG shown in Fig. 1 provided a working model for established and possible determinants of cord serum IgE relevant for this study. We considered district and year of birth, maternal age (7), ethnicity (7), maternal education (10), number of older living siblings (11), chronic or severe maternal respiratory infections during pregnancy (12), duration of labor, gestational age (13), child's gender (7), and smoking of mother or other members of the family before (14), as well as during pregnancy (7). With a high proportion of female smokers in the Czech Republic, exposures to tobacco smoke were of interest and the most relevant factor was maternal smoking during pregnancy.

Preliminary modeling revealed that many of the associations hypothesized in the DAG were, in these data, weak or non-existent. After fitting an initial full model relating PAH exposure during months 4, 5, and 6, to cord serum IgE concentration, variables were removed that did not predict the outcome with adequate precision ($p > 0.15$) and were not confounders empirically (removal changed the PR for PAHs by less than 10%).

As meteorological factors are often correlated with ambient air pollution, we attempted tight control. We examined mean temperature over 3 days prior to delivery for short-term associations and over 45 days to represent longer-term trends but neither showed any association with cord serum IgE levels (data not shown).

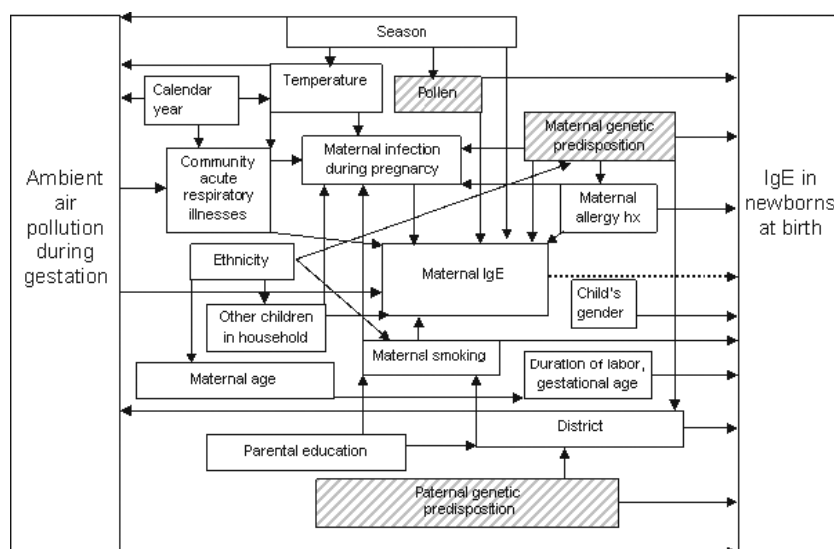


Fig. 1. Directed acyclic graph. This directed acyclic graph relates ambient air pollution to cord serum IgE levels. Boxes with a clear background represent variables measured in this study, while those with a dashed grey background were not. Dotted lines indicate that the direct relationship to the outcome is speculative and may not be causal. For instance, maternal IgE may not cause a change in cord serum IgE; instead, any association may be a result of confounding from maternal genetics.

Although temperature has not been reported to be associated with humoral immune outcomes, seasonal differences in total cord IgE levels have been (15, 16). We conjecture that these might be explained by increased pollen or mold spore levels, more hours of daylight or increased time spent outdoors, or other causes. Therefore, in further models, we adjusted for season. Because associations with fall, winter, and spring were similar, we combined them into a binary variable for season when at least 15 days of the fifth month of gestation occurred within the three summer months. The pollen season in Central and Eastern Europe varies from March to August (17, 18). Replacing summer by spring yielded similar results, and we chose the model with the lowest AIC value. The results presented in this study are from models that included district and year of birth and also, where indicated, maternal IgE and season.

We hypothesized that children with a maternal predisposition to develop atopy, for which maternal total IgE concentrations can be considered a surrogate, might have a different IgE response to pollutants (7, 10). We investigated effect modification by maternal atopic disposition [defined as maternal IgE concentration ≥ 100 IU/ml (10)] of the association between pollutants and cord IgE; thus, we report the association of pollutants on cord IgE separately for infants with atopic and non-atopic mothers. We also investigated whether the cord IgE relationship with maternal smoking differed by maternal atopy status.

All models were adjusted for the sampling design, namely stratified sampling without replacement in strata defined by: (i) district of

residence, (ii) delivery of either preterm or low birth weight infant, and (iii) year of birth. Multivariable regression models were fit with SAS[®] statistical software Version 9.2 (SAS Institute Inc., Cary, NC, USA). Results were expressed as the PR for elevated cord IgE associated with a monthly increase of 100 ng/m³ PAH exposure or a monthly increase of 25 $\mu\text{g}/\text{m}^3$ PM_{2.5}. These increments in air pollution levels were very close to two times the monthly arithmetic standard deviations during the study period, which were 53.2 ng/m³ for PAHs and 13.1 $\mu\text{g}/\text{m}^3$ for PM_{2.5}.

Results

Significantly higher proportions of births were observed in 1996 (vs. 1994 or 1995) and in Prachatice district (37% vs. 28%) in the cord IgE subset compared to the original POS cohort (Table 1). The subset with cord IgE levels resembled the larger cohort with regard to maternal age, ethnicity, smoking, and education, as well as paternal smoking and education, but included fewer mothers of high parity.

Cord serum IgE levels were below 0.35 IU/ml (the limit of detection) in 55% of the samples (Table 2). Sixteen percent of deliveries had measurable IgE in the range between 0.35 and below 0.9 IU/ml, and 29% were ≥ 0.9 IU/ml (elevated level). Among births to mothers with evidence of atopy, 61% had detectable levels of cord IgE and 46% were elevated.

Much of the local air pollution results from coal burning for home heating and in local power plants for electricity generation, contributing to higher levels of PAH and PM_{2.5} during winter

Table 1. Comparison of characteristics at birth of IgE subset (n = 459) and full cohort (n = 4386): deliveries in 1994–97, Prachatice and Teplice, Czech Republic

Characteristics	Cord IgE subset (n = 459) # (%)	Full cohort (n = 4386) # (%)
District		
Prachatice	170 (37)	1230 (28)
Teplice	289 (63)	3156 (72)
Season of birth		
Winter	122 (27)	969 (22)
Spring	130 (28)	1126 (26)
Summer	101 (22)	1210 (28)
Autumn	106 (23)	1081 (25)
Year of birth		
1994	60 (13)	1313 (30)
1995	142 (31)	1606 (37)
1996	251 (55)	1420 (32)
1997	6 (1)	47 (1)
Low birth weight (in grams)		
<2500	38 (8)	234 (5)
≥2500	421 (92)	4152 (95)
Weeks gestation at birth		
<37	47 (10)	201 (5)
≥37	412 (90)	4185 (95)
Gender		
Male	257 (56)	2296 (52)
Female	202 (44)	2089 (48)
Missing	0 (0)	1 (<1)
Mother's age at delivery		
<20	54 (12)	599 (14)
20–24.9	225 (49)	1962 (45)
25–29.9	119 (26)	1150 (26)
30–34.9	51 (11)	489 (11)
≥35	10 (2)	181 (4)
Missing	0 (0)	5 (<1)
Ethnicity of mother		
Czech/other	403 (88)	3919 (89)
Romani	56 (12)	466 (11)
Missing	0 (0)	1 (<1)
Number of livebirths		
0–1	199 (43)	1562 (36)
2	185 (40)	1268 (29)
3+	75 (16)	1549 (35)
Missing	0 (0)	7 (<1)
Number of cigarette/day smoked by mother before pregnancy		
None	284 (62)	2652 (61)
1–10	113 (25)	1084 (25)
11–20	56 (12)	591 (14)
21+	4 (1)	39 (<1)
Missing	2 (0)	20 (<1)
Number cigarettes/day smoked by father during pregnancy		
None	194 (42)	1782 (40)
1–10	127 (28)	1066 (24)
11–20	115 (25)	1175 (27)
21+	4 (1)	160 (4)
Missing	19 (4)	203 (5)
Mother's education		
Did not complete primary school	5 (1)	62 (1)
Primary school	106 (23)	913 (21)
Secondary school	204 (44)	1915 (44)
Secondary school with leaving exam	120 (26)	1219 (28)
Student	0 (0)	23 (<1)
University	24 (5)	239 (5)
Missing	0 (0)	15 (<1)

Table 1. (Continued)

Characteristics	Cord IgE subset (n = 459) # (%)	Full cohort (n = 4386) # (%)
Father's education		
Did not complete primary school	4 (1)	29 (<1)
Primary school	68 (15)	736 (17)
Secondary school	252 (55)	2256 (51)
Secondary school with leaving exam	90 (20)	958 (22)
Student	0 (0)	16 (<1)
University	31 (7)	257 (6)
Missing	14 (3)	134 (3)

(Fig. 2a,b). Median monthly PAH concentrations in the six colder months (November–April) were 40 and 80 ng/m³ for Prachatice and Teplice respectively, compared to 19 and 30 ng/m³ in the other months, with similar patterns for PM_{2.5} concentrations. Median temperatures were very similar for the two districts: 2.6°C in the cold months, 14.2°C in the warm months.

Regression results

The pattern for elevated cord serum IgE in relation to the two pollutants over the 9-month gestation period was inverse U-shaped, with lower PRs in the first 2 months and increased PRs from the fourth through seventh months (Fig. 3a,b). For example, higher average PAH or PM_{2.5} exposure in month two of gestation was associated with a PR for elevated cord serum IgE of 0.69 (95% CI: 0.50, 0.95) for a 100 ng/m³ increase in PAHs and a PR of 0.77 (0.55, 1.07) for a 25 µg/m³ increase in PM_{2.5}. Conversely, exposure during the middle of gestation was associated with a higher prevalence of elevated cord IgE. The peak second-trimester association for PAH was in the fifth month, with a PR of 1.64 (1.29, 2.08), while for PM_{2.5} it was the sixth

Table 2. IgE concentrations in cord blood samples*

IgE concentration (IU/ml)	Total study sample n (%)	Atopic mother ^{†‡} n (%)	Non-atopic mother [‡] n (%)
<0.35 [§]	251 (55)	58 (39)	193 (62)
≥0.35–<0.9	74 (16)	23 (15)	51 (17)
≥0.9	134 (29)	69 (46)	65 (21)
Total	459	150	309

*Maternal blood IgE measurements were taken in 463 deliveries; among these, 459 had cord IgE measurements.

[†]Atopic mother was defined as one whose IgE concentration was ≥100 IU/ml.

[‡]The two groups are significantly different at the 5% significance level.

[§]Limit of detection.

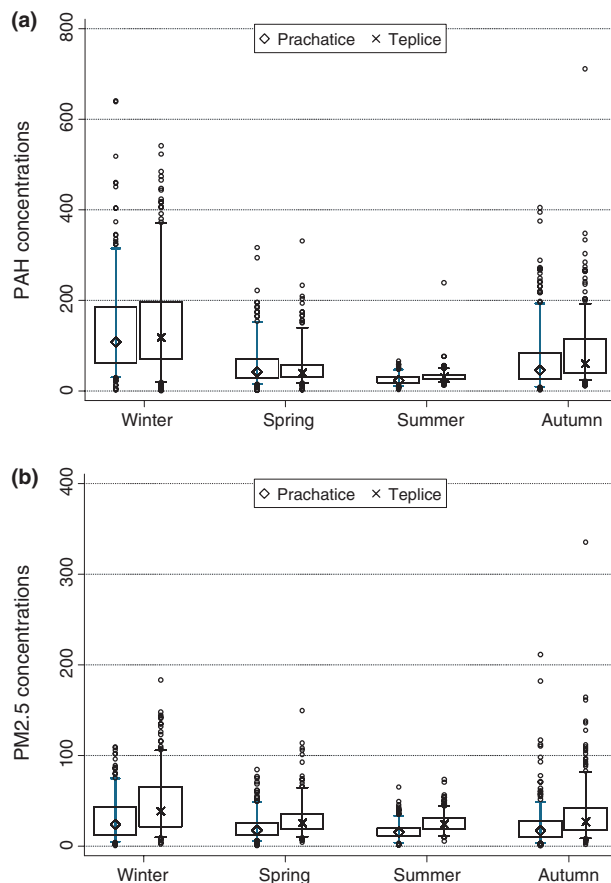


Fig. 2. Box plots of daily means of ambient polycyclic aromatic hydrocarbons (ng/m^3) (a) and $\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$) (b) levels, September 1, 1993 to January 15, 1997, by season and district in the Czech Republic. The upper and the lower limits of the box plot whiskers were set to 95th and 5th percentiles, respectively. The plots are based on daily means for the pollutants. We used monthly means in the regression models. Because of regression toward the mean, the ranges as well as the variances were reduced in the monthly means when compared to the daily means.

month with a PR of 1.66 (1.30, 2.13). Exposure during the seventh month was also associated with higher prevalence of elevated cord IgE: the PAH PR was 1.59 (1.23, 2.06) while for $\text{PM}_{2.5}$, it was 1.43 (1.05, 1.96). In general, the associations were slightly stronger for PAH than for $\text{PM}_{2.5}$; these PRs can be compared because the exposure increments were each standardized to twice the SDs for the respective pollutants.

All models adjusted for district and year of birth. Other factors did not predict cord IgE and hence did not confound the air pollution associations: maternal smoking, age, ethnicity, education, respiratory infections, number of older siblings, duration of labor, gestational age, and child's gender.

Further adjustment for maternal IgE and season of exposure did not markedly alter the

air pollution associations, which remained statistically significant (Fig. 3a,b). Use of the limit of detection as the cutpoint yielded results in the same direction (data not shown).

The association of PAH and $\text{PM}_{2.5}$ with cord IgE was stronger among infants whose mothers had low IgE levels compared with those whose mothers had serum IgE ≥ 100 IU/ml. Greater exposures to PAHs from the fourth to seventh month resulted in a significantly increased risk for elevated cord IgE exclusively in infants whose mothers were *non-atopic*, whereas no similar association was observed among those whose mothers had an atopic predisposition (Fig. 4a,b). The strongest association of PAH with elevated cord IgE was observed in the fifth month: the PR was 2.35 (1.57, 3.53) for non-atopic mothers, compared with 1.23 (0.80, 1.89) in atopic mothers. Fewer associations were seen for $\text{PM}_{2.5}$, but those PRs that were significantly different from the null were largely among non-atopic mothers (Fig. 4a,b). The direct effect of maternal atopy alone on elevated cord IgE was a twofold increase in prevalence (median PR was 2.08).

We also assessed heterogeneity of the smoking associations and found that infants of non-atopic mothers who smoked had an increased PR for elevated cord IgE of 1.56 (1.02, 2.39), while infants of atopic mothers who smoked had a PR of 0.79 (0.55, 1.12).

Discussion

In this cohort from the Czech Republic, concentrations of PAHs and $\text{PM}_{2.5}$ in ambient air were related to IgE levels in cord serum, with the direction of association dependent on the gestational window of exposure. Higher pollutant exposures during the first trimester (especially in the first 2 months of gestation) were associated with a *lower* prevalence of elevated cord IgE, whereas increased exposures in mid-pregnancy (months four to seven of gestation for PAHs, months six and seven for $\text{PM}_{2.5}$) were associated with a *higher* prevalence of elevated cord IgE. Based on an initial screen of a wide array of individual- and community-level covariates and inclusion of those that met criteria for potential confounding, the final results are unlikely to be confounded, except insofar as other pollutants that coexist in air may be associated with the ones we measured. There may be some confounding between PAH and $\text{PM}_{2.5}$, but the correlation was too high to fit a multi-pollutant model ($r = 0.75$).

The associations of these pollutants with elevated cord IgE were also found to be modified

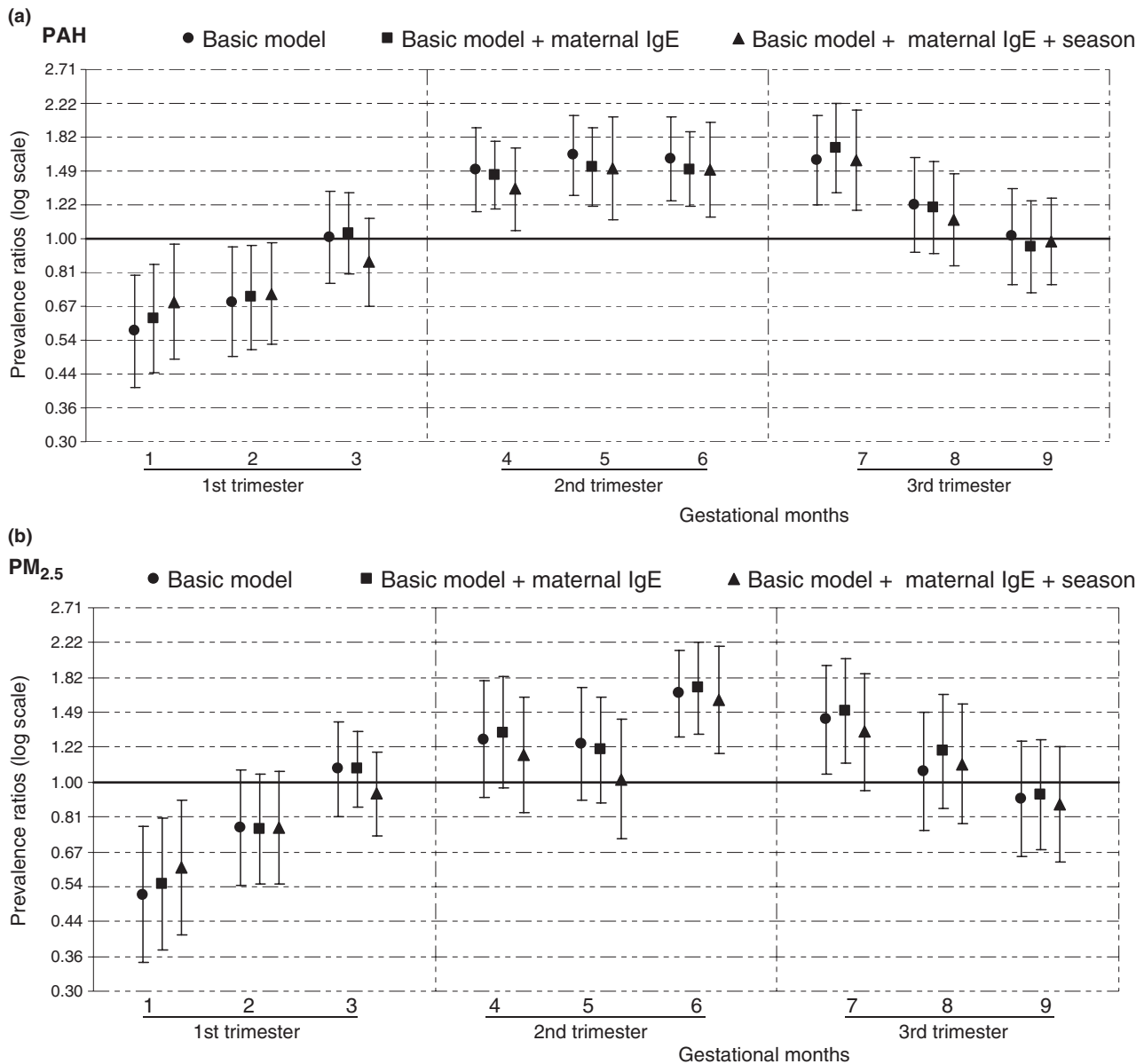


Fig. 3. Adjusted prevalence ratios (PRs) for cord serum IgE levels above threshold of 0.9 IU/ml associated with monthly gestational exposures to air pollution. The PRs are for an incremental increase of 100 ng/m³ average monthly polycyclic aromatic hydrocarbons (a) and 25 µg/m³ average monthly PM_{2.5} (b). Births were from May 1994 to mid-January 1997, Teplice and Prachatice districts, Czech Republic. Basic model was adjusted for district and year.

by maternal atopy status characterized as serum total IgE level ≥ 100 IU/ml. Elevated PRs for high cord IgE in association with these pollutants were almost exclusively limited to infants whose mothers were non-atopic. This may be because cord IgE was already elevated in infants with atopic mothers (PR of about 2.0), such that the incremental increase in risk beyond that conferred by genetic factors was relatively small. PRs for air pollution and elevated cord IgE were generally of lower magnitude than those for maternal atopy.

First trimester

In the 4 wk following implantation, i.e., roughly the second month post-LMP, production and proliferation of B lymphocytes begin. Localized IgE production starts around week 11, predominantly in the liver, followed by the spleen and lungs. This is also when priming of T lymphocytes occurs (18, 19). The lower prevalence of elevated cord IgE levels associated with higher air pollution in the first trimester is in accord with our previously published observations of an

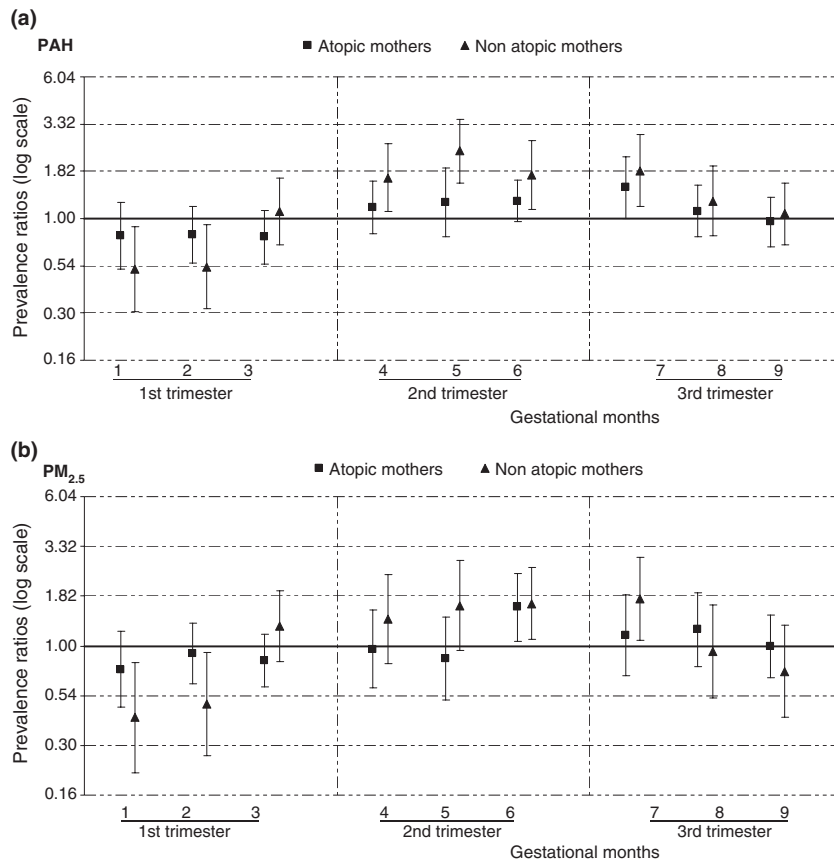


Fig. 4. Adjusted prevalence ratios (PRs) for cord serum IgE levels above threshold of 0.9 IU/ml associated with monthly averaged exposures to air pollution, separately for atopic and non atopic mothers. The PRs are for an incremental increase of 100 ng/m³ average monthly polycyclic aromatic hydrocarbons (a) and 25 µg/m³ average monthly PM_{2.5} (b). Births were from May 1994 to mid-January 1997, Teplice and Prachatice districts, Czech Republic. The models were adjusted for district, year, and season.

average decrease in the percentages of CD19⁺ (B) lymphocyte counts in relation to higher pollution levels during the same gestational windows in the same study population (20). It is possible that not only the B-cell count but also their activity is reduced. These observations suggest exposure in a critical period of embryogenesis or early fetal organogenesis may have a longer-term impact on immunologic maturation.

Second and third trimesters

The observed associations of PAH exposure during gestational months four to seven with elevated cord IgE concentrations suggest that in mid-fetal life, air pollution may stimulate B-cell production and thus enhance second trimester fetal production of circulating IgE. A similar finding has been reported by Takenaka et al. (21) who demonstrated enhanced IgE production from B cells after PAH (diesel exhaust particles) exposure in mid-gestation. Circulating endogenous fetal IgE is thought to begin between weeks 20 and 25 post-LMP, with production occurring in multiple fetal organs (21, 22). Our results suggest that air pollutant exposure around this time may influence its production. A possibly parallel finding was immune reactivity to birch

pollen at birth, which was more likely if the birch pollen season occurred in mid-gestation when compared with other time windows (18).

Although we have previously shown lymphocyte proportions to be altered in relation to air pollution exposures in the last few weeks prior to birth (5), we observed no significant association of cord IgE with exposures in the eighth and ninth months.

Covariates

Elevated cord IgE levels have also been reported in association with prenatal exposures to cigarette smoke, lead (23), and allergens derived from the mother's mattress (24). Nevertheless, the literature on prenatal exposure to cigarette smoke and IgE is not consistent: some studies report associations with higher cord serum IgE levels (7), while others do not (15, 25). Ownby et al. (25), who used urinary cotinine to determine smoking status, also found that maternal smoking did not influence cord serum IgE (or IgD) concentrations. These findings are similar to ours: in the full sample, cigarette smoke had no influence on cord IgE.

However, among those infants whose mothers were non-atopic, cigarette smoke was associated

with elevated cord serum IgE, whereas no association was observed for infants of atopic mothers. This finding is similar to a reported IgE-sensitizing effect from environmental tobacco smoke that was confined to infants of non-allergic parents (26). In contrast, another European study showed higher odds of elevated IgE in children whose mothers smoked only when one or both parents had allergies; these children were one to 10 yr of age; however, the study focused primarily on post-natal smoking (27). We have no post-natal IgE levels in the Czech study. The lack of association in neonates of atopic mothers may be because the predominant effect of maternal atopic predisposition simply swamps the influence of environmental factors. The parallelism of the heterogeneity by maternal atopy for *in utero* exposure to both cigarette smoke and ambient air pollutants in our study population is notable. PAH and PM_{2.5} are the important constituents of cigarette smoke, and hence, the similarity in findings adds to the credibility for a causal relationship.

Evidence supports heritability of atopic tendencies (28). We and others (13) observed that mothers with higher total IgE levels were more likely to give birth to children with elevated IgE levels (Table 2). Therefore, adjustment for inherited predisposition for atopy could yield more valid results, but empirically, the associations were hardly changed, indicating maternal atopy was not a confounder. However, the finding of effect measure modification dictated that adjustment for maternal atopy was necessary and the stratified results were, in fact, most informative. Although our study was not large, even in these stratified analyses, results were stable: non-atopic smoking mothers whose infants had elevated IgE constituted the smallest cell, with $n = 22$.

Gender was not predictive in our sample; most (13, 15), but not all (29), studies have found higher IgE levels in boys than in girls. Our data on maternal respiratory conditions during pregnancy were not based on medical records, and hence, the lack of association of maternal respiratory infection with cord IgE levels may be unreliable. Moreover, there is no agreement that infection during pregnancy increases cord IgE levels (11).

Cord Serum IgE measurement

Geometric means for cord serum IgE levels have been reported with different methods yielding various results. Applying the CAP system, a Japanese study reported a geometric mean of 0.286 IU/ml (30), similar to the

0.246 IU/ml geometric mean in our sample using the same method. Bergmann et al. (31) considered 36% of the infants they studied as 'sensitized' (CAP test IgE value ≥ 0.35 IU/ml); this criterion yielded 45% in our sample. The same group in a different study (7) reported that 8.5% of newborns had IgE levels of ≥ 0.9 IU/ml, when compared with 29% from our study. They also reported a geometric mean of 0.106 IU/ml; however, they assigned a value of 0.01 IU/ml for all samples below the detection limit. A Taiwanese study reported one-third of newborns to have cord IgE analyzed by CAP ≥ 0.35 IU/ml (13), whereas we observed 45% above this cutpoint. These higher fractions of detectable and elevated cord IgE levels might be attributed to environmental, dietary, or genetic factors. Although we did not test for contamination of cord blood with mother's blood, we believe that it was unlikely, as our cord serum samples were taken before delivery of the placenta on the placental part of the cord. Furthermore, sensitivity analysis excluding the highest 2.5% of values demonstrated essentially unchanged results (data not shown), lending further credence to the validity of the cord measurements.

Because of a large proportion of non-detectable cord serum IgE levels and the wide variation at the high end, we categorized cord IgE, basing cutpoints on biologic considerations. Bergmann et al. (7), who also used the CAP method to analyze cord IgE, suggested the threshold of 0.9 IU/ml (which we used) to be a useful risk indicator. When we analyzed log-transformed IgE as a continuous outcome, results were similar and again strongest in the children of non-atopic mothers (data not shown).

Potential sources of error

Errors in the estimation of LMP could have resulted from either within-woman or between-woman variability in menstrual cycle lengths, spot bleeding, faulty recall by the mother, or clinician errors in estimates of gestational age. These errors would result in an incorrect assignment of monthly exposures for some women. As these errors are unlikely to be related to cord IgE and may have misclassified the gestational ages in either direction, they most likely have attenuated the associations.

Use of monthly pollutant averages had a high probability of reducing the impact of imputation of missing PAH measurements. Only one air monitor was used in each district, which undoubtedly resulted in errors of exposure

assignment. When area-wide measures of exposure to air pollution such as those obtained from fixed-site monitoring stations are used as proxies for personal exposures, the estimates of pollutant effects were generally smaller than exposure estimates determined by personal sampling (32). Accordingly, non-differential measurement error would attenuate associations, but we are unable to assess the extent of this problem.

Relevance

Differences between the study sample and the full cohort were minimal or were controlled in the analysis, assuring generalizability of the findings to the full population in the two districts. Concentrations of organic pollutants in this investigation resembled levels reported from the United States, Asian, and other European cities; hence, the results, if caused by air pollution, may be further generalized beyond the Czech Republic (5).

To our knowledge, an investigation of this type has not been conducted for air pollution exposures, and moreover, few studies have addressed gestational time dependency for environmental factors in relation to markers of immune development. Differential associations were observed with cord serum IgE from early vs. mid-gestational exposures, underscoring the complexity of fetal immune programming. Intriguingly, first trimester exposure, when fetal IgE production begins in several developing organs, was linked to *decreased* risk for elevated IgE at delivery. Whether any of the changes we observed in association with gestational air pollution exposures lead to altered susceptibility for allergy in childhood is uncertain (19). On average, cord serum IgE levels track with serum IgE levels at 9 months of (post-natal) age (33). However, IgE concentrations increase several orders of magnitude during childhood and pre-adolescence (33), and do not appear to correlate well with allergic sensitization in childhood (27). Possibly genetic factors determine the extent to which cord serum, along with other biomarkers, can predict allergic disease in later life.

The findings of this study related to critical gestational windows argue for the need for additional research to elucidate mechanisms that might underlie these variable effects. Our results showing marked heterogeneity for both of the air pollutants and cigarette smoke, with the most sensitive infants being those whose mothers were non-atopic, emphasize the possibility of genetic or epigenetic factors in immune development in the prenatal or early childhood periods.

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