

Original article

In utero exposure to lead and cord blood total IgE. Is there a connection?

Background: Lead exposure and total immunoglobulin E (IgE) have been shown to be positively related in animals and humans even at lead levels below those recognized as toxic. In the last decades, exposure to lead has become more frequent in urban areas of industrialized as well as of developing countries where IgE-mediated allergy prevalence has also increased.

Methods: We examined for the first time the relationship between *in utero* exposure to lead and cord blood total IgE in two samples of 137 and 237 mother–newborn pairs, respectively, recruited in Paris.

Results: Cord blood IgE was positively related to hair lead level at birth, providing an integrated measure of long-term exposure *in utero*, in each cohort (Spearman's coefficient $r = 0.32$, $P < 0.001$ and $r = 0.19$, $P < 0.01$, respectively) and in the combined cohort ($r = 0.21$; $P < 0.01$). The relationship appeared to be more pronounced in newborns of nonallergic mothers ($r = 0.24$; $P < 0.01$) than in those of allergic mothers ($r = 0.12$). This could be due to the fact that familial history of allergy, the strongest determinant of IgE development, may overshadow the influence of lead on IgE in the offspring.

Conclusions: Our findings suggest a possible intervention of environmental exposure besides genetic factors in early life development of IgE production. Further studies are needed to confirm the finding.

I. Annesi-Maesano¹, R. Pollitt²,
G. King³, J. Bousquet⁴, G. Hellier⁵,
J. Sahuquillo⁵, G. Huel⁵

¹Immediate Hypersensitivity Epidemiology Department (IHED), INSERM U472: Epidemiology & Biostatistics, Villejuif, France; ²Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; ³Department of Behavioural Health, Penn State University, University Park, PA, USA; ⁴Hôpital Arnaud de Villeneuve, Montpellier, France; ⁵Department of Toxicological and Environmental Epidemiology, INSERM U472, Villejuif, France

Key words: cord blood; cotinine; early life; hair; history of allergy; IgE; *in utero*; lead; passive smoking.

Dr Isabella Annesi-Maesano
Department of Immediate Hypersensitivity
Epidemiology (DIHE)
INSERM U472: Epidemiology & Biostatistics
16, Ave P. Vaillant-Couturier
F94807 Villejuif CEDEX
France

Accepted for publication 27 January 2003

Immunoglobulin E (IgE) production is partly directed by type 2 helper T cells (Th2) (1) and an abnormal shift in the homeostatic Th1/Th2 balance towards a predominant Th2 response has been reported in allergies mediated by IgE (2). Various factors (e.g. allergenic exposures, infections, and diet) are suspected to differently influence this shift.

The recent increase in the incidence of IgE-mediated allergies in industrialized countries may be due, at least partly, to environmental toxicants found in urban areas. One of the major toxicants is lead, the concentration of which is becoming elevated due to characteristics of the overall environment in inner cities (3, 4) where, in addition, the prevalence of asthma has been found to be higher than elsewhere (5). The role of lead in the development of IgE-mediated allergy is convincingly supported by both experimental and animal studies. *In vivo*, *ex vivo*, and *in vitro* adult mouse studies have shown that lead exposure at levels far below those recognized as overtly toxic inhibits adult mouse Th1-related responses (e.g. IFN- γ production) and enhances

Th2-related responses (e.g. IL-4, IL-5, IL-10, IL-13 and IgE production) (6–9), which means that lead might be an immunosuppressive agent in animal systems. Furthermore, it has recently been observed that mouse neonates exposed to lead prenatally and/or postnatally had significantly higher plasma IgE levels than age-matched controls (10). This finding suggests that lead exposure either *in utero* or lactationally could significantly increase the risk of atopy (IgE production) in children. However, detrimental effects of lead on IgE-mediated allergy and IgE levels in humans have rarely been examined. A unique study conducted among 279 children aged 9 months to 6 years drawn from a population-based sample has shown a positive association between blood lead levels and total IgE (11). We are unaware of any studies investigating *in utero* exposure to lead in humans. Yet, early life exposure to lead might be particularly crucial in the developmental period in which the immune system hovers in the balance between Th1 and Th2.

The purpose of the present study was to investigate the relationship between *in utero* exposure to lead and cord

blood total IgE level in newborns recruited in two maternity hospitals in Paris (France). The allergic and smoking status of the mother was taken into account to estimate the relative influence of environmental exposures compared with genetic factors in IgE production.

Material and methods

Population

Two distinct subject groups recruited in two maternity hospitals in Paris served as the study populations for this study. Only mother–newborn pairs derived from single full-term births were considered in the analysis. Children are still being followed up to study their development.

Study A

The sample initially consisted of a subgroup of 799 mother–newborn pairs enrolled in a central maternity hospital of Paris (Baudelocque) between January and May 1985 in Paris. Births that took place during the weekend ($n = 170$), holidays ($n = 101$), or from 5 PM to 9 AM ($n = 271$) were excluded from the final sample. Stillbirths ($n = 33$) were also excluded. Mothers who gave birth to premature babies ($n = 7$), had Caesarean sections ($n = 36$), or who did not speak French ($n = 5$) were also excluded. Of the 176 women solicited for the study, 168 gave informed consent and eight refused; 137 mother–newborn pairs had blood total IgE assessed.

Study B

A sample of 247 Caucasian mother–newborn pairs was recruited in a maternity hospital of northern Paris (Robert Debré) between January 1991 and December 1992. Mothers having stillbirths, multiple births, pregnancies under regular drug treatment, and births with Caesarean section at a gestational age of < 37 weeks as well as newborns with acute foetal distress, AGPAR less than eight and congenital malformations were excluded. All mothers gave informed consent; 237 pairs had blood total IgE assessed.

Biological and toxicological assessments

Blood was obtained at delivery from the umbilical cord of newborns and from the vein of mothers by venipuncture using Becton Dickinson devices. Blood was initially sampled in a heparinized Vacutainer® tube (Franklin Lakes, NJ). Serum was then separated in aliquots by centrifugation. Placenta (study A only) and hair (taken from the occipital regions of newborn's and mother's heads in both studies) were also collected at birth. Blood, sera and placenta specimens were frozen at -80°C until lead, total IgE and cotinine were assessed. Hair was kept as recommended in a dry place. Assessments were performed at the end of the collection of the data in each survey by the same technicians with the same instruments.

Lead concentration was assessed in both maternal and cord blood using a flameless atomic absorption spectrophotometer with a Zeeman effect corrector (Perkin Elmer 4100 ZL, Courtaboeuf, Quebec, Canada) and was expressed in $\mu\text{g/dl}$. The method used for sampling, pretreating the hair and the placenta, and chemical analyses to assess lead concentration has been described previously (12). Lead was expressed in ppm for both blood and hair. The accuracy and reliability of lead determination were checked by national

standards of quality control. The detection limits for the techniques for lead determinations were $0.1 \mu\text{g/dl}$ in the blood and 0.05 ppm in the hair.

Total IgE were assayed in sera (but maternal serum IgE was not assessed in study A) using Pharmacia IgE Enzyme Immuno assay Ultra 50 (Pharmacia Diagnostic AB, Bois d'Arcy, France). Samples were run in duplicate. The sensitivity in cord blood was 0.01 IU/ml and in maternal blood 0.05 IU/ml . Infants (only two in study A) whose blood had been contaminated maternally, as shown by the presence of IgA, were excluded from the analysis.

Cotinine levels in maternal urine (collected within 1 day around delivery) and cord blood were used to validate maternal smoking data. Urine was frozen at -80°C until it was analysed. Cotinine levels were determined by competitive inhibition radioimmunoassay (purchased from Helen Van Vunakis, Brandeis University, Waltham, MA) in duplicate. The sensitivity in cord blood was 0.5 ng/ml and in maternal urine 6.5 ng/ml . Urinary creatinine (CCR) was determined colorimetrically (diagnostics kits, Sigma Chemical Co. St Louis, MO) and based on the reaction between CCR and sodium picrate. Urinary cotinine excretion was expressed in nanograms per milligram of CCR because 24-h urine samples were not available.

Potential risk factors and confounders

Mothers were interviewed with a standardized questionnaire on their potential history of IgE-mediated allergy (e.g. asthma, allergic rhinitis, and eczema) as well as on risk factors (e.g. education, occupation, smoking, and ethnic groups). In study B, history of IgE-mediated allergy was confirmed to be related to a significantly higher total IgE level ($P < 0.05$). Cotinine measurements were used to confirm maternal smoking status. Furthermore, medical records were examined in order to obtain obstetrical histories and descriptive characteristics of the pregnancy and infant's delivery.

Statistical methods

Cord blood total IgE were $\log_{10}$ transformed because their distribution was highly skewed. Newborn infants with total IgE levels too low to be detected by the method ($< 0.1 \text{ IU/ml}$) were ascribed a value of 0.01 IU/ml for log transformation. Results are presented in log IgE or in geometric mean according to the type of statistical analysis.

Infant hair lead level, maternal hair lead level, infant blood lead level, maternal blood lead level and placental lead level were each examined as dependent variables in the analysis.

Classical methods were used for statistical analysis (13). To examine the relationship between quantitative variables, both parametric tests (on log IgE) and nonparametric tests (Spearman's rank correlation on lead variables) were used. Furthermore, quartiles of lead concentrations were used to examine more precisely the lead–IgE relationship using a linear regression model (presented as analysis of variance results). The two samples were analysed individually and were also subsequently combined to increase statistical power. However, a linear model was applied to allow for the study effect. Data analysis included 137 mother–newborn pairs in study A, and 237 mother–newborn pairs in study B. Statistical analyses were performed using the SAS statistical software.

Results

Mean age was 29.1 and 29.3 years for respondents in study A and study B, respectively. Newborns did not

Table 1. Characteristics of the study samples

	Combined data <i>n</i> = 374	Study A <i>n</i> = 137	Study B <i>n</i> = 237
Newborns			
Sex ratio M/F	1.0 ± 0.50	0.73	1.26
Birth weight (g), mean ± SD	3331.32 ± 438.17	3242.81 ± 454.61	3383.14 ± 420.66
AGPAR at 5 min, mean ± SD	9.96 ± 0.22	9.96 ± 0.19	9.96 ± 0.24
Cord blood IgE (IU/ml), GM, (95%CI)	0.29 (0.26, 0.33)	0.33 (0.02, 6.52)	0.27 (0.25, 0.30)
Cord blood cotinine (ng/ml)	—	10.49 ± 34.26	—
Mothers			
Age (years), mean ± SD	29.30 ± 4.45	28.99 ± 5.09	29.48 ± 4.04
Allergic status (%)	33.0	35.1	33.2
Total IgE (IU/ml), GM, (95%CI)	—	—	34.89 (28.56, 42.61)
Active smokers during pregnancy (%)	24.9 ± 43.3	26.6 ± 44	24.89 ± 43
Maternal urine cotinine (CCR, ng/mg)	—	58.14 ± 172.18	—

GM: geometric mean.

Table 2. Relationship between lead variables and infant cord blood Log(IgE) levels

Lead variable	Combined data			Study A			Study B		
	<i>n</i>	mean ± SD	Spearman's correlation coefficient	<i>n</i>	mean ± SD	Spearman's correlation coefficient	<i>n</i>	mean ± SD	Spearman's correlation coefficient
Infant cord blood (µg/dl)	326	67.37 ± 47.77	−0.05	124	106.06 ± 47.78	0.11	202	38.80 ± 19.04	−0.11
Maternal blood (µg/dl)	332	96.44 ± 57.73	−0.09	135	133.02 ± 59.69	0.07	197	61.61 ± 25.11	−0.11
Infant hair (ppm)	234	1.38 ± 1.26	0.21**	45	1.74 ± 0.65	0.32***	189	1.29 ± 1.37	0.19**
Maternal hair (ppm)	360	5.16 ± 6.08	−0.04	129	6.79 ± 5.91	−0.01	231	4.05 ± 5.96	0.02

** *P* < 0.01.

*** *P* = 0.03.

differ consistently in terms of birth weight, AGPAR, maternal allergic status, or passive smoking but significantly more male infants were born to respondents in study B (Table 1).

Baseline values of total IgE and lead

Mean cord blood IgE levels did not vary significantly from one study to the other (Table 1). As expected, maternal and infant IgE were strongly correlated with each other in study B ($r = 0.38$, $P < 0.001$). In spite of the fact that total IgE has been related to ethnic group, no effect of ethnic group on cord blood IgE was found in our investigation and hence ethnic group was not considered as a confounder in the analysis. The mean lead levels for subjects in study A were considerably higher than those in study B for each lead variable measured (see Table 2). In study A, infant and maternal blood lead levels were highly correlated ($r = 0.47$, $P < 0.0001$) whereas infant and maternal hair lead levels were not. Furthermore, placental lead level was strongly correlated with both maternal blood lead ($r = 0.37$, $P < 0.0001$) and infant blood lead ($r = 0.34$, $P < 0.0001$). In study B, infant and maternal blood lead levels were also strongly correlated

($r = 0.47$, $P < 0.0001$), while maternal and infant hair lead levels were again unrelated.

Relationships between lead and cord blood total IgE levels

There was a significant correlation in both samples between infant hair lead and cord blood log IgE levels (Table 2). No significant relationship of infant blood log IgE level to the other infant lead variables was found (in particular, placental lead in study A was unrelated to cord blood total IgE ($r = 0.11$, NS)). Combining the two data sets yielded an even more significant relationship ($P < 0.0001$). This relationship was entirely confirmed by an analysis of variance between infant hair lead (four class variable) and mean log IgE levels (Table 3). The fraction of the total variation in cord blood total IgE accounted for by the fitted model was almost 10%. Furthermore, an association of borderline statistical significance ($P < 0.10$) was observed between mother blood lead and child cord blood Log(IgE) (Table 2).

Taking maternal allergic status into account showed that cord blood IgE level was significantly associated with infant hair lead overall in children of nonallergic mothers (Fig. 1). When considering the effects of passive smoking,

Table 3. Fraction of the total variation in cord log(IgE) which is accounted for by the fitted regression models in which the different lead variables are considered (ANOVA results)

Lead (divided into quartiles)	r^2 value	P -value
Combined Data		
Infant cord blood (μg/dl)	0.01	<0.0001
Maternal blood (μg/dl)	0.01	
Infant hair (ppm)	0.09	
Maternal hair (ppm)	0.01	
Study A		
Infant cord blood (μg/dl)	0.05	0.08
Maternal blood (μg/dl)	0.02	
Infant hair (ppm)	0.16	0.06
Maternal hair (ppm)	0.00	
Study B		
Infant cord blood (μg/dl)	0.01	
Maternal blood (μg/dl)	0.06	<0.005
Infant hair (ppm)	0.05	<0.02
Maternal hair (ppm)	0.01	

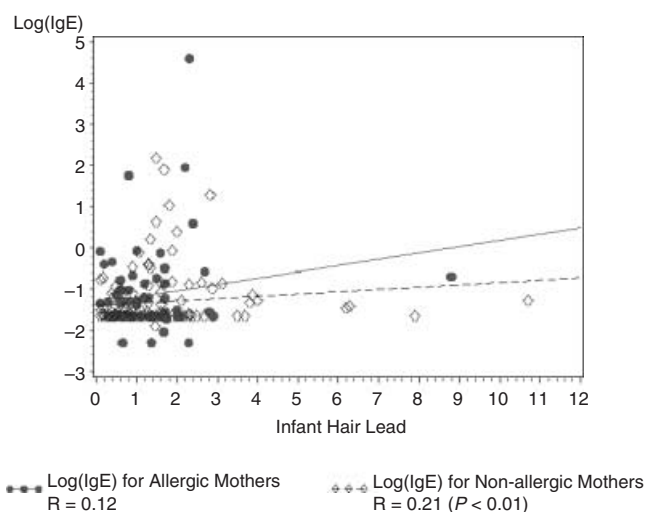


Figure 1. Infant hair lead concentration (ppm) and log (IgE) level (IU/ml) according to maternal allergic status.

no significant association was found between cotinine and infant lead variables or IgE level.

Discussion

In the present study, we show for the first time that a toxicant, lead, is related to elevated IgE at birth.

Two potential limitations of the study deserve to be discussed. The major limitation concerns the fact that only infant hair lead was significantly related to cord blood IgE. However, it has been established that hair lead provides an integrated measure of long-term exposure whereas blood lead provides a measure of short-term exposure (14). Thus, hair lead measured at birth is the best biomarker of the extent of cumulative exposure of

the foetus to lead during *in utero* life. The assessment of hair lead, which constitutes a potentially attractive measurement technique in population settings as hair samples are easy to obtain and can bioconcentrate lead, has been revealed to be reliable in various environmental studies (15). It has also been shown that hair lead is elevated when the environmental lead concentration is high (16). The second limitation concerns the samples of infants – size was small and did not include the births occurring in the night and during the weekend. The choice of a small sample size was justified by the complexity of the protocol including biological and toxicological assessments in various human specimens as hair, placenta, blood, and urine. However, the statistical power of the sample size was shown to be sufficient to detect significant differences (17). Furthermore, there is no proven evidence of selection bias in the study of the relationship between allergy and lead exposure according to the circadian rhythm of birth. That the sampling was not biased in our investigation is indirectly shown by the fact that the association between infant hair lead and cord blood IgE persisted in the different surveys at 6–7 years of difference.

Immunoglobulin E-mediated allergy is a growing and significant health problem throughout industrialized countries, particularly among children. Although it has been suggested that environmental exposures to some toxicants could have direct causal links to the rise in the prevalence of IgE-mediated allergy, these links have not been demonstrated, with the exception of environmental allergenic exposure and tobacco smoke (18). Recently, a study has pointed out the involvement of lead exposure in the production of total IgE among children (11). Our data, drawn from two different population-based samples in two separate periods of time, provide additional information as they show for the first time a statistically significant relationship between cord blood total IgE and lead exposure *in utero* as assessed by lead level in infant hair taken at birth. Placental contamination by environmental polychlorobiphenyls (PCB) and heavy metals (such as lead) through maternal blood may affect the development of the newborn and his/her immune system, by affecting normal placental functioning. In our investigation we found a borderline statistical significance between mother blood and infant hair lead, which is more likely to indicate long-term contamination of the foetus by the mother. Our findings are consistent with experimental and animal data, which indicate that lead can affect total IgE (6–9). If confirmed, such findings might lead to a diminished risk of allergy in childhood through the reduction of toxicant exposure during pregnancy.

The relationship between newborn cord blood total IgE and hair lead remains in spite of the fact that different concentrations of hair lead were observed in the two studies and actually became stronger when the data were combined, which reduces the risk that the observed relationship was caused by chance. Environmental level

of inorganic lead has diminished in France due to various preventive strategies (change of fuel, restoration of old houses, and water pipes) that became common place during the time period between the two surveys. As a consequence, the mean lead level was diminished in the second study. However, lead and IgE levels were also related in the second survey. Indeed, experimental as well as animal data show that the relationship between lead and IgE production exists also at low lead levels.

As it could be objected that IgE antibody response is under genetic control in humans (19), we studied it in two different samples and controlled for the maternal allergic status, which is an accepted marker of the existence of genetic predisposition to produce IgE. Our data showed that the relationship between hair lead and cord blood total IgE was stronger in infants of nonallergic mothers than in those of allergic mothers. This suggests that total IgE level rose in the foetus due to environmental exposure to lead independent of familial resemblance of total IgE. There are several possible explanations for the finding that the association of infant hair lead with cord blood total IgE depended on maternal allergic status. First, it cannot be excluded that the effect of *in utero* lead on cord blood total IgE was not visible in children of allergic mothers because high IgE levels provoked by familial resemblance of IgE masked it. This was confirmed by the fact that only about 10% of the total variation in cord blood total IgE was explained by *in utero* exposure to lead in our samples. A second possibility is that the IgE produced by children of allergic mothers might differ from those of children of nonallergic mothers.

Although a direct cause-effect relationship could not be examined in our study, there is no evidence supporting a direct influence of total IgE on lead concentration. On the contrary, total IgE are influenced by exogenous toxicants such as tobacco (20), diesel exhaust products (21) and, as suggested by recent experimental and human data, lead. Hence, the hypothesis can be proposed that IgE production represents a response of the organism against environmental aggression from toxicants. The underlying mechanism might involve the suppression of Th1 response by the toxicant through the reduction of

CD56 positive natural killer cells (22) which constitutes an important source of IFN- γ , which blocks IgE production.

It is becoming increasingly apparent that IgE-mediated allergy is programmed already during intrauterine life, although the precise mechanisms are unknown (23, 24). It is more likely that a complex interaction at the materno-foetal interface between genotypes and environmental events is at the origin of the development of allergy. Placental transfer of heavy metals from the mother to the foetus might influence IgE production.

In conclusion, our data support the hypothesis that *in utero* exposure to lead might be responsible for increased cord blood IgE production. Whether lead is a surrogate for other variables that are important in atopy such as westernized lifestyle and urbanization could not be answered by our study as such variables are difficult to be quantified. However, experimental as well as animal data show a direct link between lead and IgE production. Although the predictive value of cord blood total IgE in the development of allergic disease was recently debated (25), it seems that increased IgE levels at birth are associated frequently with clinical manifestations of allergy during childhood (26). As our data are unique, further studies are needed in order to confirm the observed relationship and better elucidate underlying mechanisms. With respect to public health, the implication of lead in IgE production and thus in a predisposition to IgE-mediated allergy would constitute an important step in the prevention of allergy, as it is possible to intervene on potential lead sources and diminish lead exposure, particularly in urban environments.

Acknowledgments

We thank the mothers and the children who participated in this study. This work was done while Ricardo Pollitt was the recipient of a Fogarty International Centres and Pennsylvania State University Minority International Research Traineeship. We also thank Marie-Pierre Oryszczyn, who assessed cord blood IgE as well as cotinine.

References

- PARRONCHI P, BRUGNOLO F, SAMPOGNARO S, MAGGI E. Genetic and environmental factors contributing to the onset of allergic disorders. *Int Arch Allergy Immunol* 2000;**121**:2-9.
- UMETSU DT, DE KRUYFF RH. Th1 and Th2 CD4+ cells in the pathogenesis of allergic diseases. *Proc Soc Exp Biol Med* 1997;**215**:11-20.
- WILEY JF II, BELL LM, ROSENBLUM LS, NUSSBAUM J, TOBIN R, HENRETIG FM. Lead poisoning: low rates of screening and high prevalence among children seen in inner-city agency departments. *J Pediatr* 1995;**126**:392-395.
- ROTHENBERG SJ, WILLIAMS FA JR, DELRAHIM S, KHAN F, KRAFT M, LU M, MANALO M, SANCHEZ M, WOOTEN DJ. Blood lead levels in children in south central Los Angeles. *Arch Environ Health* 1996;**51**:383-388.
- EGGLESTON PA. Environmental causes of asthma in inner city children. The National Cooperative Inner City Asthma Study. *Clin Rev Allergy Immunol* 2000;**18**:311-324.
- HEO Y, PARSONS PJ, LAWRENCE DA. Lead differentially modifies cytokine production *in vitro* and *in vivo*. *Toxicol Appl Pharmacol* 1996;**138**:149-157.

7. KISHIKAWA H, SONG R and LAWRENCE DA. Interleukin-12 promotes enhanced resistance to *Listeria monocytogenes* infection of lead-exposed mice. *Toxicol Appl Pharmacol* 1997;**147**:180–189.
8. MC CABE MJ JR and LAWRENCE DA. Lead, a major environmental pollutant, is immunomodulatory by its differential effects on CD4+ T cell subsets. *Toxicol Appl Pharmacol* 1991;**111**:13–23.
9. MILLER TE, GOLEMBOSKI KA, HA RS, BUNN T, SANDERS FS, DIETERT RR. Developmental exposure to lead causes persistent immunotoxicity in Fischer 344 rats. *Toxicol Sci* 1998;**2**:129–135.
10. SYNDER JE, FILIPOV NM, PARSON PJ, LAWRENCE DA. The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. *Toxicol Sci* 2000;**57**:87–94.
11. LUTZ PM, WILSON TJ, IRELAND J, JONES AL, GORMA, JS, GALE NL, et al. Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. *Toxicol* 1999;**134**:63–78.
12. BONITHON-KOPP C, HUEL G, MOREAU T, WENDLING R. Prenatal exposure to lead and cadmium and psychomotor development of the child at 6 years. *Neuro-behav Toxicol Teratol* 1986;**8**:307–310.
13. SNEDECOR GW COCHRAN WG. *Statistical methods* 6th edn. Univ Press, Iowa State, Ames 1997.
14. MELNICK JL COWGILL GR. Lead analyses of hair as an indication of exposure to lead. *Proc Soc Exp Biol Med* 1938;**38**:899–902.
15. BENCKO V. Use of human hair as biomarker in the assessment of exposure to pollutants in occupational and environmental settings. *Toxicology* 1995;**101**:29–39.
16. SCHUHMACHER M, DOMINGO JL, LLOBET JM, CORBELLA J. Lead in children's hair, as related to exposure in Tarragona province, Spain. *Sci Total Environ* 1991;**104**:167–173.
17. ORYSZCZYN MP, ANNESI-MAESANO I, CAMPAGNA D, SAHUQUILLO J, HUEL G, KAUFFMANN F. Head circumference at birth and maternal factors related to cord blood total IgE. *Clin Excp Allergy* 1999;**29**:334–341.
18. ANNESI-MAESANO I. Epidémiologie de l'asthme de l'enfant. *Med Ther Ped* 1999;**2**:162–168.
19. FEIJEN M, GERRITSEN J, POSTMA DS. Genetics of allergic disease. *Br Med Bull* 2000;**56**:894–907.
20. SHERRILL DL, HALONEN M, BURROWS B. Relationships between total serum IgE, atopy, and smoking: a twenty-year follow-up analysis. *J Allergy Clin Immunol* 1994;**94**:954–962.
21. TAKAFUJI S, NAKAGAWA T. Air pollution and allergy. *J Investig Allergol Clin Immunol* 2000;**10**:5–10.
22. SVENSSON BG, HALLBERG T, NILSSON A, SCHUTZ A, HAGMAR L. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int Arch Occup Environ Health* 1994;**65**:351–358.
23. ANNESI-MAESANO I, MOREAU D, STRACHAN D. *In utero* and perinatal complications preceding asthma. *Allergy* 2001;**56**:491–497.
24. STAZI MA, SAMPOGNA F, MONTAGANO G, GRANDOLFO ME, COUILLIOT M-F, ANNESI-MAESANO I. Early life factors related to clinical manifestations of atopic disease but not to skin-prick test positivity in young children. *Pediatr Allergy Immunol* 2002 **13**:105–112.
25. HIDE DW, ARSHAD SH, TWISELTON R, STEVENS M. Cord serum IgE: an insensitive method for prediction of atopy. *Clin Exp Allergy* 1991;**21**:739–743.
26. ANNESI I, ORYSZCZYN MP. Contribution of epidemiology to the study of allergic response in children. *Rev Mal Respir* 1994;**11**:325–344.