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Persistent organic pollutants and children's respiratory health: The role of cytokines and inflammatory biomarkers

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ABSTRACT

Evidence of adverse effects of persistent organic pollutants (POPs) on the developmental respiratory and immune systems in children is still limited, and the biological mechanisms behind such effects are not fully understood. The aim of the present study is to evaluate the effects of prenatal DDE, HCB and ΣPCB exposure on children's respiratory health from birth to 14 years and to evaluate the role of immune biomarkers in these associations.

We measured prenatal DDE, HCB and ΣPCB levels in 405 participants of the INMA-Menorca birth cohort (Spain) and collected information on wheeze, chest infections, atopy and asthma from birth until the age of 14 years. At age 4 years, 275 children provided serum samples and IL6, IL8, IL10, TNFα and C-reactive protein were measured. We applied linear and logistic regression models and generalized estimating equations.

Prenatal DDE was associated with wheeze at age 4 years [RR (95% CI) per doubling of concentration = 1.35 (1.07, 1.71)], but not thereafter. Prenatal HCB was associated with wheeze [1.58 (1.04, 2.41)] and chest infections [1.89 (1.10, 3.25)] at age 10 years. No associations were found with ΣPCBs. IL10 levels increased with increasing POP concentration, with HCB showing the strongest association [β (95% CI) = 0.22 (0.02, 0.41)]. IL8, IL10 and TNFα were associated with wheeze and/or chest infections and IL10 was associated with asthma.

Prenatal DDE and HCB exposure was associated with respiratory health of children at different ages. This study further suggests a possible role of IL10, but not of the other immune biomarkers examined, as an early marker of chronic immune-related health effects of POPs.

1. Introduction

Even though the production of many persistent organic pollutants (POPs), such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB) or polychlorinated biphenyls (PCBs), was banned in many countries since the 70s, these compounds can still be detected in the human population because of their capacity to bioaccumulate. Early-life exposure to POPs may adversely influence the development of the respiratory and immune systems in children (Gascon et al., 2013). A recent study including more than 4000 children from eight European birth cohort studies found increasing prenatal DDE levels to increase the risk of wheeze and/or bronchitis under the age of 18 months (Gascon et al., in press). Similar associations were also observed in a Canadian birth cohort in relation to low respiratory tract infections (Dallaire et al., 2004) and acute otitis media (Dewailly et al., 2000). This Canadian cohort also observed associations between prenatal PCB153 (Dallaire et al., 2004, 2006) and HCB (Dewailly et al., 2000) and respiratory infections. Furthermore, a recent birth cohort study including almost 900 mother–child pairs observed that prenatal exposure to HCB and PCB-118 increased the risk of suffering from asthma at the age of 20 years. Several studies have assessed the impact of early life

Abbreviations: BMI, body mass index; CRP, c-reactive protein; CV, coefficient of variation; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; ELISA, enzyme linked immunosorbent assays; GC, gas chromatography; HCB, hexachlorobenzene; IL, interleukin; INFγ, interferon gamma; INMA, Infancia y Medio Ambiente; LOD, limit of detection; LOQ, limit of quantification; PBMC, peripheral blood mononuclear cells; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; sICAM-1, soluble intercellular adhesion molecule-1; SPT, skin prick test; sVCAM-1, soluble vascular cell adhesion molecule-1; TNFα, tumor necrosis factor alpha.

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exposure to POPs on immune cell counts (i.e. T-cells or B-cells), with the aim to evaluate potential biological mechanisms of the association between POP exposure and immune and respiratory health in children. Immune cell counts are useful as general indicators of general immune status (Gascon et al., 2013), however, cytokine assays have the potential to provide more specific mechanistic insights into the effect of environmental exposures (Duramad et al., 2007; Tryphonas, 2001). Increased levels of certain cytokines and biomarkers of inflammation, including interleukin (IL) 4, IL5, IL8, and IL10, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and C-reactive protein (CRP), have been associated to asthma and related symptoms in children (Deraz et al., 2012; Figueiredo et al., 2012; Heaton et al., 2005; van de Kant et al., 2012; Robroeks et al., 2010; Tang et al., 2002). However, only four small studies in children ($N < 83$) assessed cytokine response in relation to prenatal exposure to POPs (Bilrha et al., 2003; Brooks et al., 2007; Noakes et al., 2006; Tsuji et al., 2012), and three of these measured in cord blood, a matrix where cytokine response has been shown to be very low (Holt and Jones, 2000; Krampera et al., 2000). Therefore, larger studies measuring cytokines later in childhood are required. In the INMA (Infancia y Medio Ambiente) birth cohort study of Menorca, including more than 400 children, increasing prenatal exposure to DDE during pregnancy was found to be associated with children's wheeze at age 4 years and asthma at the age of 6.5 years (Sunyer et al., 2005, 2006). No associations with prenatal HCB and PCBs were observed. Looking for potential mechanisms, no associations were found between prenatal DDE and total cell and eosinophil counts or specific IgE.

Because the long-term respiratory health effects of prenatal exposure to POPs has only been assessed in one study (Hansen et al., 2013) and because there is lack of information on the mechanisms behind the respiratory health of POPs, the present study aims to evaluate the effects of prenatal DDE, HCB and ΣPCB exposure on children's respiratory health, including chest infections and asthma related symptoms, from birth to 14 years of life and to evaluate the role of cytokines and biomarkers of inflammation in these associations.

2. Methods

2.1. Study population

The INMA-Menorca birth cohort (Spain) recruited women presenting for antenatal care between 1997 and 1998 (Guxens et al., 2012). A total of 482 mothers (94% of those eligible) were enrolled into the cohort. Of these, 405 provided information on the respiratory health of their children in the 1st year of life and had information on POP levels in cord blood (study population A). At the age of 4 years, blood samples were drawn from 360 children and stored at −20 °C. Due to budget limitations, cytokines and biomarkers of inflammation were measured in 275 serum samples, which were selected randomly from individuals with complete information on prenatal POP exposure and wheeze and chest infections at age 4 years (study population B).

2.2. Exposure assessment

Cord blood samples were collected and analyzed for DDE, HCB and PCB101, 118, 138, 153 and 180. Analyses were carried out in the Department of Environmental Chemistry of the Institute of Environmental Assessment and Water Research (IDAEA-CSIC) in Barcelona, Spain, using gas chromatography (GC) with electron capture detection (Hewlett–Packard 6890N GC-ECD; Hewlett–Packard, Avondale, PA, USA) and GC coupled to chemical ionization negative-ion mass spectrometry (Hewlett–Packard 5973 MSD) (Carrizo et al., 2007). PCB congeners 101, 118, 138, 153 and 180 were summed to create one single variable (ΣPCBs).

2.3. Respiratory health

The occurrence of wheeze, chest infections and asthma was evaluated via interviewer-led questionnaires with the mother. Wheezing was reported in questionnaires at years 1, 2, 3, 4, 6.5, 10 and 14, and was described as, "whistling or wheezing from the chest, but not noisy breathing from the nose". At the age of 2, 3, 4, 6.5 and 10 years parents were asked about chest infections: "In the last 12 months, did your [child] have a chest infection?". At the age of 6.5 years both the question on wheeze and chest infection referred to the last 24 months. At ages 10 and 14 years, parents were asked if their child had ever been diagnosed of having asthma by a doctor. Also, at the age of 6.5 years atopy status of the children was tested using skin prick test (SPT). A positive skin test to at least one allergen (Der p 1, Der f 1, cat, dog, grass pollen, mixed tree, mixed graminae, parietaria) was considered indicative of atopy. A weal of 2 mm or greater in the presence of a positive histamine control and a negative uncoated control constituted a positive skin test (Polk et al., 2004).

2.4. Immune biomarkers: cytokines and biomarkers of inflammation

Multiplex assays provide multiple advantages in front of older techniques, such as individual enzyme linked immunosorbent assays (ELISA), because they allow to measure multiple analytes from the same sample simultaneously, which results into the use of less sample and analyses at a lower cost (Loo et al., 2011). However, this technique has also some limitations. For instance, the performance of the multiplex assay decreases with increasing number of analytes (Chaturvedi et al., 2011). Since standard cytokine panels can include a lot of analytes, and since detection of these analytes can be very low and with a high coefficient of variation (CV) between duplicates, we performed a first pilot study including 35 samples (in duplicates) in order to select the best analytes in terms of detectability and CV. These analyses were performed at Merck Millipore's laboratory in Abingdon, UK. Interferon gamma (INFγ), IL1β, IL2, IL4, IL5, IL6, IL8, IL10, IL13 and tumor necrosis factor alpha (TNFα) were analyzed in the standard MPXHCYTO-60K Millipore's panel. We chose this panel because it includes a wide spectrum of interleukins and biomarkers related to inflammation and alteration of the immune system. After the pilot study, we decided to analyze IL2, IL8, IL10, and TNF-α because the other analytes were detected in less than 10% of the samples. Further, we performed an ELISA test (R&D systems HS600b panel), with better detection rates than the multiplex technique, to measure IL6. sICAM-1 and sVCAM-1 were measured with Millipore's panel HCVD2MAG-67K. Finally, CRP was measured by immunoturbidimetry at the laboratory of the groups EGEC and CARIN of IMIM Foundation, Barcelona, Spain. All analyses were performed in duplicates except for IL6 ELISA, for which only 35 samples were tested in duplicates spread along the different plates. For CRP, those samples with values over 1 mg/dL were repeated to ensure that results were correct. IL2 was detected in less than 80% of the samples, so it was excluded from the analyses.

2.5. Other variables

Questionnaires administered to mothers during pregnancy and the subsequent follow-ups collected information on maternal and paternal asthma, rhinitis, eczema, smoking during pregnancy and during postnatal life of the child, education and social class (using the UK Registrar General's 1990 classification according to parental occupation by ISCO88 code), maternal age at birth, parity (first child or more), number of siblings (none or one or more), age of the child at the time of starting daycare attendance, duration of breastfeeding and age of the child at the time of outcome assessment. Gender, gestational age and birth weight were extracted from clinical records and maternal body mass index (BMI) during pregnancy (first trimester) and child BMI at different ages were calculated from the weight and height measurements with