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Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants

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ABSTRACT

Background: To explore possible markers of developmental immunotoxicity, we prospectively examined 56 children to determine associations between exposures to methylmercury and persistent organic pollutants since birth and the comprehensive differential counts of white blood cells (WBC) at age 5 years.
Materials and methods: Extended differential count included: neutrophils, eosinophils, basophils, lymphocytes (includingT cells, NK cells, and B cells), and monocytes. Organochlorine compounds (OCs) including polychlorinated biphenyls (PCBs) and pesticides, five perfluoroalkyl substances (PFASs), and total mercury (Hg) were measured in maternal (n = 56) and children's blood at 18 months (n = 42) and 5 years (n = 54). We constructed latent functions for exposures at three different ages using factor analyses and applied structural equation models adjusted for covariates.
Results: Prenatal mercury exposure was associated with depleted total WBC, especially for lymphocytes, where a one standard deviation (SD) increase in the exposure was associated with a decrease by 23% SD (95% CI: −43, −4) in the cell count. Prenatal exposure to OCs was marginally associated with decreases in neutrophil counts. In contrast, the 5-year PFASs concentrations were associated with higher basophil counts (B = 46% SD, 95% CI: 13, 79). Significantly reduced subpopulations of lymphocytes such as B cells, CD4-positive T helper cells and CD4 positive recent thymic emigrants may suggest cellular immunity effects and dysregulation of T-cell mediated immunity.
Conclusion: Developmental exposure to environmental immunotoxicants appears to have different impacts on WBC counts in childhood.

1. Introduction

Exposure to environmental pollutants may significantly affect the immune system and cause immunologic suppression in children [1]. The developing immune system appears to be highly sensitive to toxic effects, and experimental animal studies and in vitro assays have shown immunomodulatory effects of persistent organic pollutants (POPs), especially polychlorinated biphenyls (PCBs) [2–5], organochlorine pesticides (OCPs) [5–7], and perfluoroalkyl substances (PFASs) [8–10], as well as mercury and other metal compounds [11,12]. These immunotoxicants are ubiquitous, most are persistent, and they are widely detected in children and adults from the general population [13].

In humans, recent epidemiologic studies have reported immunomodulatory effects of mercury [14–16] and POPs [13,17,18] in children and adults. For example, higher exposures to PFASs were associated with a reduction of the humoral immune response to booster vaccination [17–19], whereas mercury exposures were associated with increased pro-inflammatory cytokines, antinuclear and antinucleolar autoantibodies and decreased anti-inflammatory cytokines [20]. Developmental exposure to PCBs and OCPs has also been shown to modulate the immune system in infants [21,22], children [23], and adults [13]. Higher 6-month infant concentrations of PCB-153 and DDE (dichlorodiphenyldichloroethylene) were strongly associated with

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lower 6-month BCG-specific antibody levels [24], and higher maternal and infant PCB concentrations have been associated with a reduced volume of the infant thymus, the site of T-cell maturation [25]. Moreover, early-life environmental PCB exposure was associated with fluctuations in major lymphocyte subsets [26], and affected the dynamics of cell surface lymphocyte receptor expression [27]. Overall, previous studies provide compelling evidence of the detrimental effects of exposure to environmental toxicants on the immune system, including immune cell counts, cytokine responses, and levels of specific antibodies [28]; However, most of the human studies on immunotoxicity of POPs and mercury lack a prospective design with age-related exposure profiles, and no study has so far attempted to examine the potential joint effect of different POPs on the immune system development. These complexities also indicate a need for new statistical tools to disentangle the role of multiple exposures on multiple immune system markers [29].

The white blood cell (WBC) count has been proposed as an immunologic end point to detect immunotoxic effects of environmental contaminants in prospective epidemiologic studies [30]. In humans, WBC counts have proven useful in signaling clinically relevant hematologic changes that may result in clinically identifiable immune disorders [31], with absolute numbers of WBC providing biologically more reliable information than percentages [32].

In the present study, we explored the associations between exposures to POPs and methylmercury measured at birth and at ages 18 months and 5 years in regard to extended WBC differential counts at age 5.

2. Materials and methods

2.1. Study population

The present study focuses on fifty-six children from a cohort of consecutive births formed in the Faroe Islands to include 490 children during an 18-month period between late 2007 and early 2009. Whole blood was taken from the cord and from the mother two weeks after parturition. In addition, maternal hair at parturition was also sampled. Blood and hair were obtained from the child at successive clinical examinations at ages 18 months and 5 years. At age 5 years, children underwent detailed examinations including immune system biomarkers. On dates when fresh blood samples could be transported to the immunological laboratory in Denmark, we obtained maternal consent for a subgroup of 56 children to draw additional blood for the purposes of the present study. Standard questionnaires were used to record past medical history, current health, social factors, and nutritional habits during and before pregnancy. Relevant obstetric information, including birth weight, parity and maternal age were abstracted from hospital's medical records.

The study protocol was approved by the ethical review committee serving the Faroe Islands and by the institutional review board at the Harvard T.H. Chan School of Public Health, and written informed consent was obtained from all mothers.

2.2. WBC counts and lymphocyte subsets

The total number of WBCs, neutrophils, eosinophils, lymphocytes, and monocytes were recorded by standard procedures using the ABX Pentra DX 120 (Horiba, United Kingdom/Germany). Furthermore, T-cell (CD3), T-helper cells (CD4), T-cytotoxic cells (CD8), B-lymphocytes (CD19), NK (CD16/56) cells and CD4+ recent thymic emigrants (CD4-RTE) absolute counts were performed by a single-platform no-lyse-no-wash procedure. Fifty μl EDTA anticoagulated peripheral blood were incubated in TRUCount tubes (BD Biosciences, Denmark) with a panel of conjugated monoclonal antibodies. The following combinations of antibodies were used to characterize T cells as CD4T cells CD3-PerCP (clone SK7), CD4-FITC (clone SK3), CD8T cells (CD3-PerCP (clone SK7), CD8-PE (clone SK1)) and CD4-RTE (recent thymic immigrant) cells as CD3-ECD (clone UCHT1), CD4-PC7 (clone SFCT12T4D11), CD31-PE (clone WM59), CD45RA-FITC (clone L48), and CD45RO-PC5 (clone UCHL1), NK cells as CD45-PerCP (clone 2D1), CD16/56-PE (clone B73.1 + NCAM16.2), and CD3-FITC (clone SK7), and B cells as CD19-PE (clone 4G7), CD45-PerCP (clone 2D1) (BD Biosciences, Beckman Coulter and AbD Serotec, Denmark). The samples were measured on FC500 flow cytometer (Beckman Coulter, Denmark). The laboratory participates in the quality assurance program by National External Quality Assessment Site (NEQAS).

2.3. Exposure assessment

PCBs, OCPs (*p,p'* and *o,p'* isomers of dichlorodiphenyltrichloroethane [DDT] and DDE, and hexachlorobenzene [HCB]) and PFASs (perfluorohexane sulfonic acid [PFHxS], perfluorooctanoic acid [PFOA], perfluorooctane sulfonate [PFOS], perfluorononanoic acid [PFNA], and perfluorodecanoic acid [PFDA]) were measured in maternal (n = 56) and children's serum at ages 18 months (n = 42) and 5 years (n = 54). In addition, as measures of methylmercury exposure, mercury (Hg) was measured in cord blood, maternal blood and hair, and in child blood and hair at age 5 years.

Serum analyses of DDE, DDT, HCB, and PCBs were carried out by the same procedure for all samples using a gas chromatograph with electron capture detection at the Department of Environmental Health, University of Southern Denmark [23]. To avoid problems with PCB congeners not assessed or below the detection limit, ΣPCB was calculated as the sum of major congeners 138, 153, and 180 multiplied by 2 [33]. DDE at 18 months and 5 years, and DDT at 18 months were detected in less than 50% of samples and were therefore not further considered. The PFASs were measured using online solid-phase extraction and analyzed using high-pressure liquid chromatography with tandem mass spectrometry [34]. The accuracy and reliability of the data was ensured by including, in each analytical series, quality control serum samples, calibration standards, and reagent and serum blanks. Within-batch and between-batch coefficient of variations were better than 3.0% and 5.2% for all analytes. Total mercury analyses in hair and blood were performed on a Direct Mercury Analyzer (DMA-80, Milestone Inc, Sorrisole, Italy), with imprecision below 4%. All POP results below the limit of detection (LOD) were replaced by the LOD divided by $\sqrt{2}$.

2.4. Statistical analyses

WBC, Hg, and POP concentrations were all log-transformed (base 2) before they entered the models to approximate a normal distribution. One child had very high WBC count (Total = 16.4×10^9 cells/L) due to a fever and was consequently removed from the analyses. Due to the large number of exposure variables, we used structural equation models (SEM) to assess the covariate-adjusted associations between prenatal, 18-month, and 5-year exposures and the extended WBC counts at 5 years.

We first conducted an exploratory factor analysis (EFA) without a priori information on the structure and correlations in the data, followed by a confirmatory factor analysis (CFA) to categorize exposures into a small number of factors, thereby reducing the extent of multiple comparisons. For prenatal exposures, this method resulted in three factors explaining 65% of the variance. Factor 1 included the 5 PFASs, factor 2 included mercury indicators (i.e., cord blood, maternal blood and hair concentrations), and factor 3 included HCB, PCB, *p,p'*-DDE, *o,p'*-DDT, and *p,p'*-DDT. For