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Prenatal exposure to polychlorinated biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood

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ABSTRACT

We investigated whether prenatal exposure from the maternal diet to the toxicants polychlorinated biphenyls (PCBs) and dioxins is associated with the development of immune-related diseases in childhood. Children participating in BraMat, a sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa), were followed in the three first years of life using annual questionnaires (0–3 years; $n = 162$, 2–3 years; $n = 180$), and blood parameters were examined at three years of age ($n = 114$). The maternal intake of the toxicants was calculated using a validated food frequency questionnaire from MoBa. Maternal exposure to PCBs and dioxins was found to be associated with an increased risk of wheeze and more frequent upper respiratory tract infections. Furthermore, maternal exposure to PCBs and dioxins was found to be associated with reduced antibody response to a measles vaccine. No associations were found between prenatal exposure and immunophenotype data, allergic sensitization and vaccine-induced antibody responses other than measles. Our results suggest that prenatal dietary exposure to PCBs and dioxins may increase the risk of wheeze and the susceptibility to infectious diseases in early childhood.

1. Introduction

Food items may contain environmental toxicants like the organochlorines polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs, commonly called dioxins). PCBs and dioxins are highly lipophilic and accumulate in the food chain. The diet is assumed to be the main source of human exposure to these substances, providing more than 90% of the total exposure (Domingo and Bocio, 2007).

Studies have shown that PCBs and dioxins cross the placenta and reach the foetus (Covaci et al., 2002; Suzuki et al., 2005). Prenatal exposure to immunotoxicants is of concern since the foetus may be especially vulnerable due to an extensively developing immune system (Holsapple et al., 2004; van Loveren and Piersma, 2004). Adverse effects on the immune system may result in immune-related diseases like allergy, asthma and autoimmune conditions, or increased susceptibility to infectious diseases.

In the Norwegian birth cohort BraMat, we have previously reported that prenatal dietary exposure to PCBs and dioxins is associated with an increased risk of wheeze and infections during the first year of life (Stolevik et al., 2011). The present study aimed to investigate if immunotoxic effects of prenatal dietary exposure to PCBs and dioxins change or persist into early childhood by performing a three-year follow-up of the children participating in the birth cohort BraMat. Associations between maternal dietary exposure, determined from food frequency questionnaires (FFQ), and measures of the child's immune function were investigated. In addition to questionnaire data, blood parameters were examined:

Abbreviations: BMI, body mass index; Bw, body weight; dl-PCBs, dioxin-like PCBs; ELISA, enzyme-linked immunosorbent assay; FFQ, food frequency questionnaire; FSC, forward scatter; Hib, *Haemophilus influenzae* type b; IU, international unit; MBRN, the medical birth registry of Norway; MoBa, the Norwegian Mother and Child Cohort Study; ndl-PCBs, non-dioxin-like PCBs; PAU, Phadia arbitrary unit; PCBs, polychlorinated biphenyls; SSC, side scatter; TEQ, toxic equivalents; URTI, upper respiratory tract infections.

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concentrations of allergen specific IgE antibodies, vaccine-induced antibody levels and immunophenotype data including regulatory T cells.

An advantage of using FFQ data are that the results may indicate how exposure to toxicants from the diet affects the health of the children, and how the results may relate to the intake of food and what food items contribute the most to exposure. This information can be valuable when counselling women of fertile age regarding their diet.

2. Materials and methods

2.1. Study population

The BraMat cohort ($n = 205$) has previously been described in Stolevik et al. (2011). In short, in 2007–2008 invitations (~800) were sent to pregnant women already enrolled in the Norwegian Mother and Child Cohort Study (MoBa). MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health, including 108.000 children from all over the country born between 1999 and 2008 (Magnus et al., 2006). The recruitment rate in BraMat was 25%. The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics and the Data Inspectorate. All mothers gave their written informed consent both to MoBa and BraMat.

2.2. Blood sampling and handling

Venous blood was collected from the three year old children (age mean (range), 36 (33–43) months) either at their doctor's office, at home by a technician, or at a commercial laboratory (Fürst medical laboratory). Blood was collected into BD Vacutainer®SST™II serum gel separation tubes with butterfly blood collection sets (Becton, Dickinson and Company (BD), Franklin Lakes, NJ, USA). The blood was allowed to clot for at least 30 min before centrifugation at 1000–1300*g* for 10 min at room temperature. Aliquots of the serum samples were stored at −20 °C until further analyses. Blood was also collected into EDTA BD Vacutainers® (BD) and kept at room temperature until analyses were performed.

2.3. Determination of vaccine responses

As a measure of the immune function of the children, antibody responses to four of the vaccines in the Norwegian Childhood Vaccination Program were examined. Two viral (attenuated measles and rubella), one bacterial toxin (tetanus toxoid) and one bacterial polysaccharide-protein conjugated vaccines (*Haemophilus influenzae* type b (Hib)) were selected. Vaccines against tetanus and Hib are generally given at age 3, 5 and 12 months, and measles and rubella at age 15 months.

2.3.1. Vaccine antibody titers

The serum samples were analysed for anti-measles IgG antibodies using Enzygnost Anti-Measles Virus IgG ELISA (Siemens, Marburg, Germany) and for anti-rubella IgG antibodies using Serion Rubella Virus IgG ELISA (Virion/Serion, Würzburg, Germany). Both assays were performed as recommended by the manufacturers. The antibody levels were given as OD-values. Concentrations of specific IgG antibodies to tetanus toxoid (Simonsen et al., 1986), and Hib (modified from Phipps et al. (1990)) were determined using ELISA techniques. The antibody levels to tetanus toxoid was given as IU/ml. Values below the detection limit (<0.1 IU/ml) were given the value 0.07 IU/ml (2/3 of the detection limit). Regarding the anti-Hib assay, microtiter plates were coated with Hib oligosaccharide conjugated to human albumin (1 mg/ml) (NIBSC, Potters Bar, UK). The serum samples were diluted twofold starting at dilution 1:10. Goat anti-human IgG-alkaline phosphatase (ALP) (Sigma–Aldrich, St. Louis, MO, USA) was used as conjugate. The concentration of specific antibodies was calculated using human anti-Hib capsular polysaccharide serum as standard (LOT No. 1983; FDA, Silver Spring, MD, USA).

2.4. Determination of allergen specific IgE

The serum samples were analysed for allergen-specific IgE antibodies using ImmunoCAP Phadiatop® Infant (Phadia AB, Uppsala, Sweden) containing 11 allergens selected to be relevant for young children. The concentrations of antibodies were expressed as Phadia Arbitrary Units/l (PAU/l) indicating the degree of sensitisation. Children with antibody concentrations ≥0.35 PAU/l were according to the manufacturer's recommendation considered to be sensitised.

Sera from the children found to be sensitised using Phadiatop Infant, were analysed for specific IgE antibodies to 'individual' allergen preparations from house dust mite, cat, dog, hen's egg, cow's milk, peanut, timothy and birch. The concentrations of antibodies were expressed as kU/l, and children with antibody concentrations ≥0.35 kU/l were according to the manufacturer's recommendation considered to be sensitised. The analyses were performed as recommended by the manufacturer.

2.5. Immunophenotyping by flow cytometry

Within 24 h, immunophenotyping was performed on whole blood samples according to the protocol of the manufacturer (BD). In short, the blood samples were stained with the antibodies in the Multitest 6-colour TBNK reagent and Pacific Blue-labelled anti-CD14 (BD) for 15 min in the dark at room temperature. Red blood cells were lysed. The cells were counted using a BD LSRII flow cytometer with BD FACSDiva Software version 6.1.2. The lymphocytes were gated based on side light scatter (SSC) and CD45 expression, and monocytes were gated based on CD14 and CD45 expression. The percentage and the absolute number of the following lymphocyte subsets were determined: T-lymphocytes ($CD3^+$), T-helper cells ($CD3^+CD4^+$), cytotoxic T-lymphocytes ($CD3^+CD8^+$), B-lymphocytes ($CD19^+$), natural killer (NK) cells ($CD16^+CD56^+$) and natural killer T-lymphocytes ($CD3^+CD16^+CD56^+$). Absolute numbers of the leukocytes (cells/μl) were determined by the use of Truecount tubes (BD).

For assessment of regulatory T cells, the blood samples were stained with the antibodies in the Human Regulatory T cell Coctail (FITC-labelled anti-CD4, PE-Cy7-labelled anti-CD25, Alexa647-labelled anti-CD127; BD) for 30 min in the dark at room temperature. After lysing of red blood cells, the cells were washed twice and centrifuged at 200g for 5 min at 18 °C, and resuspended in 400 μl washing buffer. The percentage of regulatory T cells was determined using a four steps gating strategy as recommended by the manufacturer. Finally, regulatory T cells were determined as percentage $CD127^{low}$ $CD25^{high}$ cells of $CD4^+$ cells.

2.6. Assessment of exposure to the dietary toxicants

Maternal intake of the dietary toxicants PCBs and dioxins was calculated from a validated food frequency questionnaire (FFQ) used in MoBa, which was administered to the participants in the 22nd week of gestation. Description and validation based on nutrients of the FFQ is given elsewhere (Meltzer et al., 2008; Brantsaeter et al., 2008). The FFQ covers the dietary intake of the mothers during the first five months of pregnancy. The method for estimation of exposure to PCBs and dioxins has been described in Kvalem et al. (2009). In brief, an extensive database was compiled comprising all available concentrations of dioxin and PCB congeners in Norwegian food from 2000 to 2006. Intake of PCBs and dioxins was calculated by multiplying food consumption (g/day) by levels of toxicants in the food, using the FoodCalc software (http://www.ibt.ku.dk/jesper/foodcalc). Calculated intake of PCBs and dioxins from the diet has been found to be correlated with blood concentrations (e.g. dioxins and dioxin-like PCBs (dl-PCBs): $\rho = 0.34$, $p = 0.017$) (Brantsaeter et al., 2008; Kvalem et al., 2009). The diet may change from before to during pregnancy and should be considered when exposure to persistent toxicants such as PCBs and dioxins is calculated. Unpublished data from MoBa, however, suggest that 70–80% of the women do not alter their intake of fish (important source of PCBs and dioxins) from before to during pregnancy. Furthermore, Crozier et al. (2009) reported no change in fish consumption.

PCBs and dioxins can be divided into two groups according to their toxicological properties: (1) dioxins and dl-PCBs and (2) non-dioxin-like PCBs (ndl-PCBs). Similar toxicological properties of dioxins and dl-PCBs allow the combined exposure to be expressed as toxic equivalents (TEQ) (van den Berg et al., 2006). The exposure was expressed relative to the mother's self-reported body weight (bw) before pregnancy, thus the exposure to dioxins and dl-PCBs was expressed as pg TEQ/kg bw/day and exposure to ndl-PCBs as ng/kg bw/day.

2.7. Health outcomes from questionnaires

When the children in the BraMat cohort were one, two and three years of age, a questionnaire was sent to the mothers. The mothers had the choice of filling in the questionnaires and returning them by regular mail, or to give the answers by a telephone interview. The questionnaires covered topics related to the child's infectious diseases, allergy, asthma and other chronic diseases, and the use of medications. Concerning infectious diseases, the mothers were asked if the child had experienced the following diseases/complaints, and how many episodes: colds and other upper respiratory tract infections, otitis media, pneumonia, episodes of gastroenteritis with vomiting or diarrhoea and urinary tract infection. The mothers were also asked if the child had experienced any children's diseases, such as chicken pox and exanthema subitum (roseola infantum). Concerning allergy, asthma and other chronic diseases, the mothers were asked: "Has the child been diagnosed with asthma, asthma bronchitis, or bronchial hyperreactivity by a doctor? Has the child had periods of more than 10 days with dry cough, chest tightness or wheeze, or shortness of breath (hereafter called wheeze)? Has the child had eczema or itching in the face or at joints (e.g. the groin, hollow of the knee, ankle, elbow and wrist)? Has the child been diagnosed with atopic dermatitis by a doctor? Has the child been diagnosed with allergy by a doctor? Has the child any other chronic disease?"

Data for all three years merged (0–3 years of age) and data for the last year only (2–3 years of age) were investigated. Regarding the data for all three years merged and the binary health outcomes (yes/no answers), a positive answer in one or more of the annual questionnaires was noted as a "yes" in our database. Furthermore, the sum of the number of reported incidences for all three years was used for health outcomes with numbers of episodes.