In Vitro Activation of Cord Blood Mononuclear Cells and Cytokine Production in a Remote Coastal Population Exposed to Organochlorines and Methyl Mercury

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Remote coastal populations that rely on seafood for subsistence often receive unusually high doses of organochlorines and methyl mercury. Immunosuppression resulting from prenatal exposure to organochlorines has been reported in wildlife species and humans. In this study, we assessed lymphocyte activation and associated cytokine secretion in 47 newborns from a remote maritime population living on the Mid and Lower North Shore regions of the St. Lawrence River (Québec, Canada; subsistence fishing group) and 65 newborns from nearby urban settings (reference group). Cord blood samples were collected for organochlorine and mercury analyses and also to isolate cord blood mononuclear cells (CBMCs) for the *in vitro* assessment of cytokine production and expression of surface markers after mitogenic stimulation ($CD4^+CD45RO^+$, $CD8^+CD45RO^+$, $CD3^+CD25^+$, and $CD8^+HLA\text{-}DR^+$). Blood mercury and plasma concentrations of polychlorinated biphenyls (PCBs), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p,p′*-DDE), and hexachlorobenzene (HCB) were significantly higher in the subsistence fishing group than in the reference group ($p < 0.001$). No difference was observed between the two groups regarding subsets of lymphocytes showing markers of activation. *In vitro* secretion of cytokines by CBMCs after mitogenic stimulation was lower in the subsistence fishing group than in the reference group ($p < 0.05$). Moreover, we found an inverse correlation between tumor necrosis factor-α (TNF-α) secretion and plasma PCB, *p,p′*-DDE, and HCB concentrations ($p < 0.05$). Our data support a negative association between TNF-α secretion by CBMCs and prenatal organochlorine exposure. If the relationship between organochlorine and TNF-α secretion is causal, it would suggest a role for this important proinflammatory cytokine in mediating organochlorine-induced immunotoxicity in infants developmentally exposed to these compounds. *Key words:* Canada, cytokines, immune system, maternal exposure, methyl mercury, neonate, organochlorine insecticides, polychlorinated biphenyls. *Environ Health Perspect* 111:1952–1957 (2003). doi:10.1289/ehp.6433 available via *http://dx.doi.org/* [Online 29 August 2003]

Organochlorines (OCs) are a group of chemicals that includes pesticides and their metabolites [e.g., dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichlorethylene (DDE)], industrial compounds and by-products of various industrial processes [e.g., hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs), and polychlorodibenzofurans (PCDFs)]. After their release into the environment, OCs are transported by long-range atmospheric and oceanic currents to remote northern regions of the globe (Barrie et al. 1992; Macdonald et al. 2000). Once trapped in these cold regions, their lipophilic nature and resistance to biodegradation lead to their accumulation in fatty tissues of organisms and biomagnification in food chains (Dewailly et al. 1993; Ryan et al. 1997). Mercury emitted by waste incinerators and coal-fired electrical power plants can also be carried over long distances by atmospheric currents to reach northern regions. In lakes and oceans, mercury from both natural (geologic origin) and anthropogenic sources is converted by bacteria to methyl mercury, which can reach high concentrations in predator species from aquatic food chains (Muir et al. 1999).

The Lower North Shore of the St. Lawrence River, a remote coastal region of Québec, includes 15 communities spread over a 400-km shoreline extending from Kegaska to Blanc-Sablon (Figure 1). A large proportion of the 6,000 residents rely on fishing for subsistence and consequently consume large amounts of seafood (Dewailly et al. 1992). Several surveys conducted since 1990 have revealed that this population is highly exposed to PCBs (Dewailly et al. 1992; Muckle et al. 1998), mercury (Laliberté et al. 1992; Rhainds et al. 1999), and dioxin-like compounds (Ryan et al. 1997), compared with the southern Québec population. The Mid North Shore region, located closer to the towns of Sept-Îles and Port-Cartier and accessible by road, also has fish-eating communities.

Several OCs are immunotoxic in animals and humans, especially the potent 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally related compounds such as the non-*ortho*-chloro-substituted and mono-*ortho*-chloro-substituted PCBs and the 2,3,7,8-chloro-substituted PCDDs/PCDFs (Safe 1990, 1994). Studies in laboratory and wildlife animals have shown that the maturation of the immune system is especially vulnerable to the adverse effects of dioxin-like compounds and other immunotoxic OCs (Barnett et al. 1987; Holladay and Blaylock 2002; Holladay et al. 1991; Ross et al. 1997). Prenatal exposures to high doses of PCBs and PCDFs have induced immunosuppression and increased susceptibility to infectious diseases in Taiwanese infants born to Yu-cheng mothers (Rogan et al. 1988). Alterations in immune system function and increased incidence of infections have also been encountered in infants of populations environmentally exposed to OCs (Dewailly et al. 2000; Weisglas-Kuperus et al. 1995, 2000).

We recently investigated several parameters of cord blood lymphocyte function in newborns from the Lower and Mid North Shore regions of the St. Lawrence River and newborns from a reference group with lower exposure to environmental contaminants. Compared with the reference group, the subsistence fishing group showed decreases in the proportion of naive helper T-cells ($CD4^+CD45RA^+$) in umbilical cord blood and T-cell proliferation response after mitogenic stimulation (Belles-Isles et al. 2002). Moreover, the proportion of $CD4^+CD45RA^+$ cells was inversely correlated to cord blood mercury and plasma PCB concentrations, whereas T-cell clonal expansion was negatively correlated to plasma PCBs and *p,p′*-DDE concentrations.

The decrease in the proportion of naive helper T-cells noted in our previous study might indicate an improper maturation of these cells into memory cells. In this study we hypothesized that cellular activation and associated cytokine secretion are impaired by prenatal OC exposure in newborns among this

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fish-eating population. We measured membrane markers of T-cell activation (CD4/CD45RO, CD8/CD45RO, CD8/HLA-DR, CD3/CD25) and *in vitro* cytokine secretion [interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α)] after mitogenic stimulation of cord blood immune cells obtained from newborns of the fish-eating population and newborns from a reference group. Because the diet of our study population is based on marine food, we also determined plasma concentrations of omega-3 polyunsaturated fatty acids (n-3 PUFAs), because these fatty acids are known to exert beneficial effects on the regulation of the immune system (Blok et al. 1996; Harbige 1998; Hardardottir and Kinsella 1992) and to modulate cytokine production (Blok et al. 1996; Endres 1996; Harbige 1998).

Materials and Methods

Populations and sampling. The study population included pregnant women who had resided for at least 5 years in communities of the Mid North Shore and Lower North Shore of the St. Lawrence River and includes both Caucasian and Native people. Women were recruited upon admission for delivery at the Sept-Îles regional hospital between June 1997 and December 1998. We sought informed consent from all women, who were asked to complete a questionnaire on their lifestyle habits and anthropometric characteristics. Forty-seven women were recruited from the Mid and Lower North Shore of the St. Lawrence River and constituted the subsistence fishing group. The reference group contained 65 women who were residents of two small towns, Sept-Îles and Port-Cartier. The participation rate was 85% for the reference group and 60% for the subsistence fishing group. Standard anthropometric measurements were performed on newborns at birth. The protocol was approved by the medical ethical committee of the Laval University Medical Center (Québec, Canada).

Blood samples (~30 mL) were collected from the umbilical vein using a heparinized syringe, after the umbilical cord was severed, and transferred in vacutainers containing heparin. An aliquot of fresh blood was promptly sent to the immunology laboratory within 24 hr of sampling. Another whole-blood aliquot was set aside for mercury analysis. The rest of the sample was centrifuged and the plasma transferred in glass vials prewashed with hexane. Plasma and blood samples to be analyzed for OCs and mercury were stored at –20°C until time of analysis at the Laboratoire de Toxicologie/INSPQ (Québec, Canada). Five samples were rejected because of hemolysis. In other cases, sample volume was too low to conduct all immune tests, which led to the variation in the number of subjects from one test to another.

OC analysis in cord plasma. Concentrations of 14 PCB congeners (International Union for Pure and Applied Chemistry nos. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) and 11 chlorinated pesticides and metabolites [aldrin, α-chlordane, γ-chlordane, *cis*-nonachlor, HCB, *p,p′*-DDE, *p,p′*-DDT, mirex, oxychlordane, *trans*-nonachlor, and β-hexachlorocyclohexane (β-HCH)] were determined by high-resolution gas chromatography. Plasma samples (2 mL) were extracted with an ammonium sulfate:ethanol:hexane (1:1:3) solution, cleaned on Florisil columns, and analyzed on an HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual Ni-63 electron-capture detectors (Ultra-1 and Ultra-2; Hewlett Packard, Palo Alto, CA, USA). Peaks were identified by their relative retention times obtained on the two columns, using a computer program developed in-house. Quantification was performed mainly on the Ultra-1 column. Limits of detection (LODs) are 0.02 μg/L for PCB congeners and most pesticides and metabolites, except for *p,p′*-DDT and β-HCH (0.03 μg/L). We previously reported quality control procedures as well as accuracy and precision for OCs analyses in plasma (Rhainds et al. 1999). The Laboratoire de Toxicologie/INSPQ is accredited by the Canadian Association for Environmental Analytical Laboratories.

Because OCs distribute mainly in body fat, we reported their concentrations in plasma samples on a lipid basis (micrograms per kilogram lipids). Concentrations of total cholesterol, free cholesterol, and triglycerides in plasma samples were determined using standard enzymatic procedures. A commercial kit (Wako Pure Chemical Industries, Richmond, VA, USA) was used to determine phospholipids concentrations, according to the enzymatic method of Takayama et al. (1977). We estimated the concentration of total lipids in plasma using the formula developed by Phillips et al. (1989). Concentrations of n-3 and n-6 PUFAs were determined in the plasma phospholipids fraction by the Lipid Analytical Laboratory at the University of Guelph (directed by Dr. Bruce J. Holub). Plasma samples were extracted using a chloroform/methanol mixture, and the resulting lipid extracts were applied onto thin-layer chromatography plates to isolate the phospholipid fraction. Fatty acids were then methylated and their concentrations in plasma phospholipids determined by capillary gas-liquid chromatography.

Mercury analysis in cord blood. We used cold vapor atomic absorption spectrometry to measure total mercury concentration in blood samples. Samples were digested with nitric acid, and mercury was reduced by adding anhydrous stannous chloride and cadmium chloride. Metallic mercury was volatilized and detected by atomic absorption spectrometry (model 120; Pharmacia Mercury Monitor, Piscataway, NJ, USA). The LOD for blood mercury analysis is 1 nmol/L. We previously reported quality control procedures as well as accuracy and precision data for blood mercury analysis (Rhainds et al. 1999).

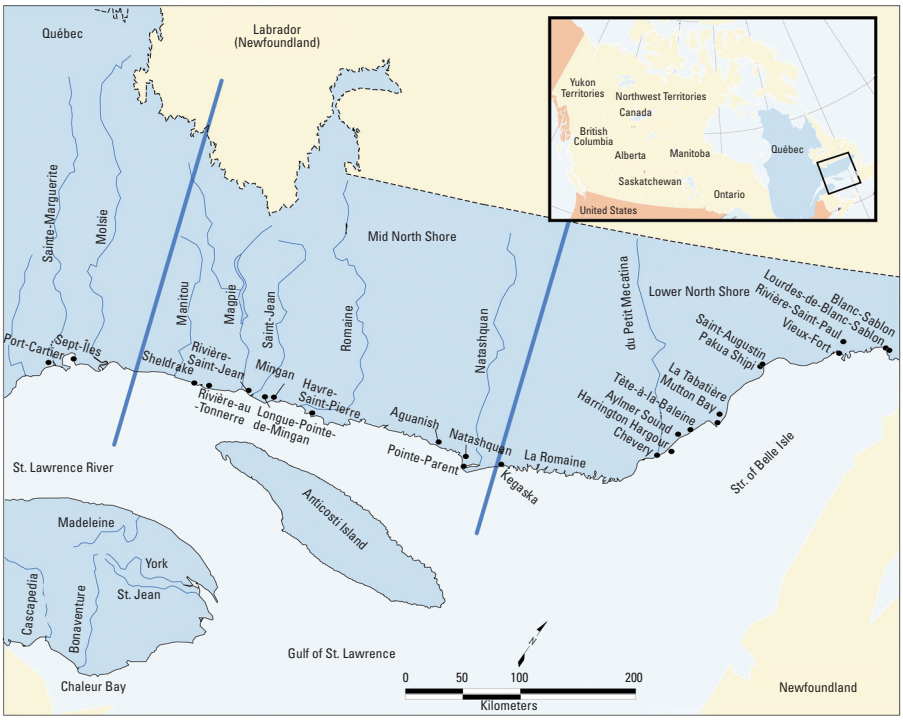

Figure 1. Location of the Mid and Lower North Shore regions of the St. Lawrence River.

Immunologic parameters. Cord blood mononuclear cells (CBMCs) were obtained after Ficoll-Hypaque density gradient centrifugation, washed, and suspended at 1×10^6 cells/mL in culture medium consisting of RPMI and 7.5% fetal calf serum supplemented with antibiotics.

Analysis of T-lymphocyte phenotypes. CBMCs (4×10^6) were incubated in triplicate at 37°C, 5% CO_2 for 72 hr with or without phytohemagglutinin (PHA; 1.25 µg/mL). CBMCs were suspended in PBS-NaN_3, and T-lymphocyte subsets were phenotyped by dual-color flow cytometric analysis using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies (Coulter, Hialeah, FL, USA). Briefly, we incubated 3–5 × 10^5 cells in 100 µL of phophate-buffered saline (PBS)-NaN_3 at 4°C for 15 min with optimal concentrations of antibodies: *a*) memory helper T cells: CD4-FITC/CD45RO-PE; *b*) memory cytotoxic T cells: CD8-FITC/CD45RO-PE; *c*) activated cytotoxic T cells: CD8-FITC/HLA-DR–PE; *d*) activated T cells bearing the IL-2 receptor: CD3-FITC/CD25-PE; and *e*) isotypic controls IgG-FITC and IgG-PE. After two washes, the cell pellets were fixed with paraformaldehyde and suspended in PBS-NaN_3 for analysis on a flow cytometer (Profile II; Coulter). Fluorescence intensity was measured (log scale) on 5,000 lymphocytes gated by scattering properties. The instrument performance was standardized daily using Immunocheck and Flow-set calibration beads (Coulter). We used quadrature analysis for dual staining to determine the proportion of double positive events (quadrant 2). Differentiated/memory $CD4^+$ or $CD8^+$ lymphocytes were expressed as the percentage of the total $CD4^+$ or $CD8^+$, respectively. Similarly, $CD8^+$ cells expressing the HLA-DR antigen were expressed as the percentage of total $CD8^+$ cells, and $CD3^+$ cells bearing IL-2 receptors were expressed as the percentage of total $CD3^+$ cells.

***In vitro* cytokine production.** CBMCs were incubated in triplicate at a concentration of 2×10^5 cells/well in the presence of PHA (1.25 µg/mL) or culture medium (negative control), at 37°C during 48 hr (IL-10 secretion) or 72 hr (TNF-α secretion). Cytokine concentrations were measured in culture supernatants by enzyme-linked immunoadsorbent assay (ELISA) after centrifugation (R&D Systems, Minneapolis, MN, USA). LODs of the assays are 7.8 pg/mL for IL-10 and 15.6 pg/mL for TNF-α.

Statistical analysis. Concentrations of contaminants and immune parameters displayed log-normal distributions. Therefore, we performed statistical analyses using $\log_{10}$-transformed values and presented geometric means in descriptive statistics. We limited statistical analyses to contaminants potentially immunotoxic that were detected in more than 50% of the samples. For contaminants and cytokines, a concentration equal to half the LOD was assumed for samples with concentrations below the LOD. We used the Student's *t*-test to compare mean values between groups (continuous variables) and the Fisher exact test to compare proportions (categorical variables). Relations between immune system parameters and contaminants were first assessed using Pearson correlation coefficients. To identify potential confounders, we tested variables that were statistically different between the subsistence fishing group and the reference group for associations with immunologic parameters, using either a Student's *t*-test (ethnicity, smoking during pregnancy) or Pearson correlation coefficients (maternal weight, n-3 PUFAs). Relationships between exposure variables and immunologic parameters were further investigated using multiple linear regression analyses to control for potential confounders. The level of statistical significance was set at 0.05. We used the SAS for Windows statistical software package (version 8.02; SAS Institute, Cary, NC, USA) to perform all statistical analyses.

Results

Forty percent of women from the subsistence fishing group were Natives, and the rest of the participants were Caucasians (Table 1). Cigarette smoking was nearly twice as prevalent in the subsistence fishing group than in the reference group. The mean body weight of women in the subsistence fishing group was 21% higher than that of women in the reference group. There was no statistically significant difference between the subsistence fishing group and the reference group with regard to gestational age, newborn weight, and sex ratio. Concentrations of n-3 and n-6 PUFAs in cord plasma phospholipids were slightly lower in the subsistence fishing group compared with the reference group (by 15 and 4%, respectively).

Only contaminants detected in more than 50% of the cord blood samples are listed in Table 2. Mean (geometric) concentrations of *p,p′*-DDE, HCB, and PCBs (sum of 14 congeners) in the subsistence fishing group were, respectively, 1.5, 1.6, and 2.5 times higher than those in the reference group. Mean blood mercury concentration was 1.8 times higher in the subsistence fishing group than in the reference group.

Proportions of cord blood T lymphocytes $CD4^+$, $CD8^+$, and $CD3^+$ expressing surface markers indicative of cell activation are shown for both study groups in Table 3. Values obtained with unstimulated and PHA-stimulated cells are presented. No statistically significant difference was observed between the subsistence fishing group and the reference group for all the markers studied, either on stimulated or on unstimulated cells. Ratios of stimulated to unstimulated cells were also computed and were not different between groups (data not shown). In addition, no correlation

Table 1. Population characteristics.

Characteristics	Subsistence fishing group ($n = 47$)	Reference group ($n = 65$)	p-Value[a]
Mothers			
Age (years; mean ± SD)	26.0 ± 4.9	27.3 ± 4.7	0.166
Ethnicity (% Caucasian)	60	100	< 0.001
Weight (kg; mean ± SD)	74.4 ± 16.9	61.6 ± 1.3	< 0.001
Gestation length (weeks; mean ± SD)	39.2 ± 1.5	39.2 ± 1.3	0.905
% Smokers	66	37	0.004
Newborns			
Sex (% male)	40	58	0.084
Weight (g; mean ± SD)	3,493 ± 486	3,455 ± 479	0.682
n-3 PUFA (% total plasma phospholipids; mean ± SD)	5.9 ± 1.3	6.9 ± 1.4	< 0.001
n-6 PUFA (% total plasma phospholipids; mean ± SD)	28.1 ± 3.0	29.2 ± 1.6	0.019

[a] p-Value obtained by Student's *t*-test for means or Fisher exact test for percentages.

Table 2. Concentrations of OCs (µg/kg plasma lipids) and mercury (nmol/L) in umbilical cord blood samples [geometric mean (95% CI)].

	Subsistence fishing group ($n = 47$)	Reference group ($n = 65$)	p-Value[a]
Organochlorines			
PCB-138	36 (27–48)	11 (9–12)	< 0.001
PCB-153	50 (36–68)	14 (12–16)	< 0.001
PCB-180	21 (15–28)	6 (5–7)	< 0.001
∑PCB-138, PCB-153, PCB-180	107 (79–146)	31 (28–35)	< 0.001
∑PCBs[b]	197 (155–250)	80 (75–86)	< 0.001
p,p′-DDE	144 (114–182)	84 (73–96)	< 0.001
HCB	14 (12–16)	9 (8–10)	< 0.001
Mercury	9.0 (7.3–11.0)	5.4 (4.6–6.2)	< 0.001

95% CI: 95% confidence interval.
[a] p-Value for Student's *t*-test applied to $\log_{10}$-transformed values. [b] Sum of 14 PCB congeners.

was noted between activated surface markers and plasma OCs or blood mercury concentrations (data not shown).

Levels of cytokines spontaneously released by CBMCs *in vitro* were similar in both groups (Figure 2). After PHA stimulation, CBMCs of newborns from the subsistence fishing group secreted less TNF-α (nearly 2-fold lower; $p < 0.05$) than did those of neonates from the reference group. IL-10 secretion also appeared to be lower in the subsistence fishing group than in the reference group, but the difference was not statistically significant (Figure 2).

Results of correlation analyses between PHA-induced cytokine production and food-chain contaminants are presented in Table 4. Negative correlations were observed between TNF-α secretion and plasma lipid concentrations of PCBs, *p,p´*-DDE, and HCB ($p < 0.05$). Although all correlation coefficients were negative, no statistically significant correlation was observed between IL-10 levels and food-chain contaminants.

For several neonates, levels of cytokines released by unstimulated cells were above those released by stimulated cells. Levels of IL-10 secreted by PHA-stimulated CBMCs did not exceed those spontaneously released for seven neonates from the subsistence fishing group and four neonates from the reference group (16% vs. 8%; Fisher exact test, $p = 0.529$). Levels of TNF-α produced by stimulated CBMCs were below basal levels for 16 neonates from the subsistence fishing group and five neonates from the reference group (36% vs. 13%; Fisher's exact test, $p = 0.022$). Stimulated-to-unstimulated TNF-α concentration ratios are shown in Figure 3 for participants of both study groups. A statistically significant negative correlation was observed between this ratio and *p,p´*-DDE plasma concentration. A similar but slightly weaker correlation was noted with total PCB plasma concentration (Pearson's $r = 0.219$, $p = 0.051$).

Because the subsistence fishing group and the reference group differed with regard to ethnic composition, maternal body weight, smoking during pregnancy, and n-3 PUFA concentrations in umbilical cord plasma phospholipids, additional statistical analyses were conducted to assess possible relations of these variables to the immunologic parameters. None of these variables were linked to the expression of surface markers or cytokine production after CBMC activation (data not shown). The inclusion in multivariate models of smoking, n-3 PUFAs, maternal weight, or ethnicity as independent variables did not modify the relation plasma OC concentrations to the secretion of TNF-α by activated CBMCs (data not shown).

Table 3. *In vitro* expression of activation markers on PHA-stimulated and unstimulated cord blood lymphocytes.

	Subsistence fishing group		Reference group		
T-cell subset[a]	Mean[b] (95% CI)	*n*	Mean[b] (95% CI)	*n*	*p*-Value[c]
$CD4^+CD45RO^+$					
PHA(–)	6.8 (5.3–8.6)	33	5.7 (4.4–7.3)	50	0.346
PHA(+)	72.2 (66.1–78.9)	34	77.4 (72.8–82.2)	51	0.195
$CD8^+CD45RO^+$					
PHA(–)	3.6 (2.7–4.8)	33	3.6 (2.8–4.6)	49	0.957
PHA(+)	68.8 (62.7–75.6)	34	72.3 (67.5–77.4)	52	0.402
$CD3^+CD25^+$					
PHA(–)	4.5 (3.4–6.0)	33	4.0 (3.3–4.9)	50	0.501
PHA(+)	74.6 (68.6–81.1)	34	75.6 (69.3–82.4)	50	0.842
$CD8^+HLA\text{-}DR^+$					
PHA(–)	1.5 (1.1–1.9)	26	1.7 (1.5–2.1)	46	0.286
PHA(+)	3.5 (2.5–4.8)	32	3.1 (2.5–4.0)	50	0.636

95%CI: 95% confidence interval.
[a]Differentiated/memory $CD4^+$ or $CD8^+$ lymphocytes are expressed as the percentage of total $CD4^+$ or $CD8^+$ cells, respectively. Similarly, $CD8^+$ cells expressing the HLA-DR antigen are expressed as the percentage of total $CD8^+$ cells, and $CD3^+$ cells bearing IL-2 receptors are expressed as the percentage of total $CD3^+$ cells. [b]Geometric mean. [c]Student's *t*-test applied to $\log_{10}$-transformed values. For calculations, a 0.1% value was attributed to "0" values (only for $CD8^+HLA\text{-}DR^+$).

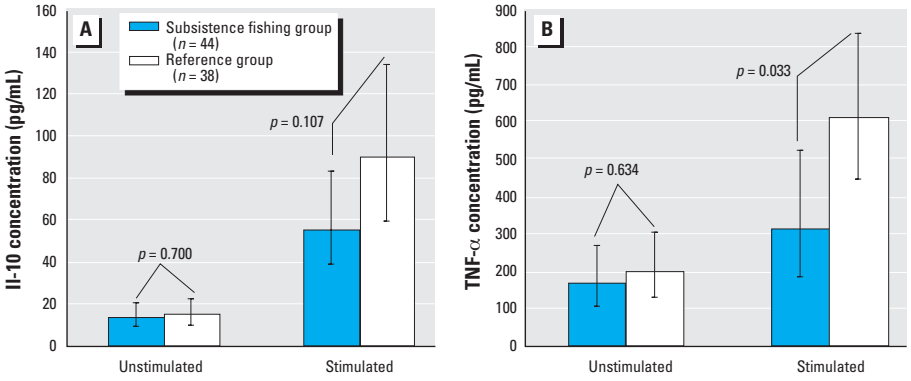

Figure 2. Concentrations of (*A*) IL-10 and (*B*) TNF-α released *in vitro* by unstimulated and PHA-stimulated CBMCs. Each bar represents the geometric mean ± SE of the mean for 44 neonates from the subsistence fishing group and 38 neonates from the reference group. Cytokine concentrations in the culture supernatant were measured by an ELISA test (pg/mL). *p*-Values are for Student's *t*-test applied to $\log_{10}$-transformed values.

Table 4. Correlations between *in vitro* production of cytokines by activated lymphocytes and either blood mercury or plasma OC concentrations in participants from both study groups.

	IL-10 (*n* = 83)		TNF-α (*n* = 83)	
	Pearson's *R*	*p*-Value	Pearson's *R*	*p*-Value
Organochlorines				
∑PCBs[a]	–0.144	0.195	–0.260	0.018
p,p´-DDE	–0.162	0.143	–0.289	0.008
HCB	–0.165	0.136	–0.284	0.009
Mercury	–0.124	0.268	–0.148	0.186

[a]Sum of 14 PCB congeners.

Discussion

In this study we investigated whether or not the *in vitro* activation of CBMCs was altered in neonates from a subsistence fishing group who received an unusually high transplacental exposure to food-chain contaminants such as OCs and methyl mercury. We reported previously that prenatal exposure to OCs was associated with a decrease in naive cells in umbilical blood samples collected from neonates from the same population (Belles-Isles et al. 2002). This led us to suspect a possible defect in the maturation of naive cells to memory cells. In the present study, we observed no difference in the proportions of cord blood lymphocytes that express surface markers indicating T-cell activation. However, *in vitro* TNF-α release by PHA-stimulated cells was lower in the subsistence fishing group than in the reference group. Furthermore, we noted a negative correlation between plasma concentration of the major OCs (PCBs and *p,p´*-DDE) and the *in vitro* secretion of TNF-α by activated CBMCs.

We could not find any study in the literature that investigated associations between prenatal exposure to food-chain contaminants, cord blood lymphocyte activation, and resulting cytokine secretion. Svensson et al. (1994) compared the expression of the activation marker $CD3^+/CD25^+$ in T cells obtained from 23 adult males with a high consumption of fish from the Baltic Sea and 20 males with virtually no fish consumption, after *in vitro* mitogenic stimulation. Similar to our results,

no difference was found between groups in the number or the proportion of $CD3^+/CD25^+$ cells after *in vitro* concanavalin A activation, even though the fish-eating group had a much higher body burden of OCs (PCBs, PCDDs/PCDFs, DDT) and mercury than did the control group (Svensson et al. 1994). Hence, prenatal or postnatal exposure to food-chain contaminants such as OCs and mercury does not appear to modify the differentiation, the maturation, or the proliferation of T cells.

Cultured CBMCs from most neonates in our study spontaneously secreted detectable amounts of TNF-α, but none exhibited signs of infection. Spontaneous secretion of TNF-α by cultured CBMCs from healthy neonates has been previously reported in the literature (Soboslay et al. 1999; Zhao et al. 2002). Although basal TNF-α secretion was similar in both groups, PHA-stimulated secretion was lower in the subsistence fishing group than in the reference group. When results were expressed as the ratio of TNF-α concentration in the supernatant of PHA-stimulated CBMCs to that of unstimulated CBMCs (Figure 3), one can readily observe that for several neonates (16 from the subsistence fishing group and five from the reference group), the stimulation of CBMCs by PHA did not increase secretion of TNF-α above levels spontaneously released by unstimulated cells. The significance of these observations is not clear at the present time. Further studies are planned with mononuclear cells from healthy donors to investigate the time course of cytokine production (basal and mitogen induced), in the presence of different OCs in the culture medium.

PCBs, *p,p′*-DDE, and HCB were all negatively correlated to mitogen-induced TNF-α secretion by CBMCs. There is some evidence in the literature that PCBs can reduce the release of TNF-α by activated immune cells. Ahne and Jarre (2002) exposed *in vitro* human blood samples to either 50 or 500 pg/μL concentrations of PCB-77 or PCB-126, two non-*ortho* PCB congeners that display dioxin-like activity. After mitogenic stimulation, blood samples treated with 50 pg/μL of either one of these congeners secreted 66% less TNF-α than did control untreated blood samples. Treatment with the 500 pg/μL concentration reduced TNF-α secretion by 93% compared with controls. These results suggest that the negative association observed in our study between some OCs and TNF-α secretion by activated CBMCs could be caused by dioxin-like PCB congeners. Binding of dioxin-like compounds to the aryl hydrocarbon receptor, a transcription factor, can antagonize the effects of another transcription factor, nuclear factor (NF)-κB, which is an important regulator of immune and inflammatory gene expression (O'Neill 2001).

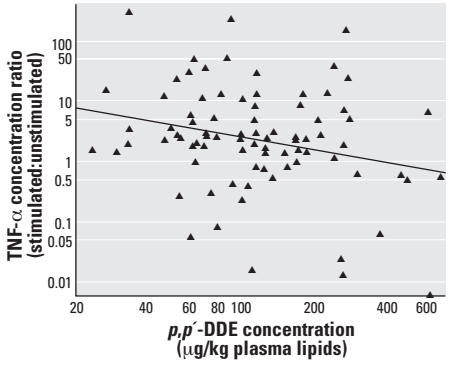

Figure 3. Ratio of TNF-α concentration in supernatant of PHA-stimulated CBMCs to that of unstimulated CBMCs. Data for all neonates are presented (n = 83). Pearson's correlation coefficient was calculated using $\log_{10}$-transformed values.

The subsistence fishing group and the reference group differed markedly regarding ethnicity and smoking habits. Additional statistical analyses revealed no relation between these factors and immune parameters measured in the present study. Concentrations of n-3 and n-6 PUFAs in plasma phospholipids were slightly higher in the reference group than in the subsistence fishing group. n-3 PUFAs are biomarkers of fish consumption, and concentrations measured in plasma phospholipids indicate that both groups were high fish consumers. It was reported that a diet rich in n-3 PUFAs may exert beneficial effects on the regulation of the immune system and autoimmune diseases (Blok et al. 1996; Harbige 1998; Hardardottir and Kinsella 1992) and to modulate cytokine production such as TNF-α, IL-1β, and IL-6 (Blok et al. 1996; Endres 1996; Harbige 1998). In our study, correlation analyses did not reveal any association between n-3 PUFAs and the immune parameters evaluated.

In summary, our results support a negative association between *in vitro* production of TNF-α and prenatal exposure to some OCs. TNF-α is a very important proinflammatory cytokine that participates in the induction of immune responses to infectious agents and possesses direct antiviral activity (Larrea et al. 1996; Nokta et al. 1991; Wong et al. 1988). An altered production of this cytokine could cause a major imbalance in the immune response and, in turn, health problems in this population, such as autoimmune diseases and increased susceptibility to infections. Additional studies are needed to explore the possible role of this important proinflammatory cytokine in mediating OC-induced immunotoxicity in infants developmentally exposed to these compounds.

References

Ahne W, Jarre T. 2002. Environmental toxicology: polychlorinated biphenyls impair TNF-alpha release *in vitro*. J Vet Med A Physiol Pathol Clin Med 49:105–106.

Barnett JB, Barfield L, Walls R, Joyner R, Owens R, Soderberg LS. 1987. The effect of *in utero* exposure to hexachlorobenzene on the developing immune system of BALB/c mice. Toxicol Lett 39:263–274.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, et al. 1992. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 122 :1–74.

Belles-Isles M, Ayotte P, Dewailly É, Weber JP, Roy R. 2002. Cord blood lymphocyte function in newborns from remote maritime population exposed to organochlorines and methylmercury. J Toxicol Environ Health 65:165–182.

Blok WL, Katan MB, van der Meer JW. 1996. Modulation of inflammation and cytokine production by dietary (n-3) fatty acids. J Nutr 126:1515–1533.

Dewailly É, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. 2000. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ Health Perspect 108:205–211.

Dewailly É, Ayotte P, Bruneau S, Laliberté C, Muir DC, Norstrom RJ. 1993. Inuit exposure to organochlorines through the aquatic food chain in Arctic Quebec. Environ Health Perspect 101:618–620.

Dewailly É, Laliberté C, Sauvé L, Ferron L, Ryan JJ, Gingras S, et al. 1992. Sea-bird egg consumption as a major source of PCB exposure for communities living along the Gulf of St-Lawrence. Chemosphere 25:1251–1255.

Endres S. 1996. n-3 Polyunsaturated fatty acids and human cytokine synthesis. Lipids 31(suppl):239–242.

Harbige LS. 1998. Dietary n-6 and n-3 fatty acids in immunity and autoimmune disease. Proc Nutr Soc 57:555–562.

Hardardottir I, Kinsella JE. 1992. Increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. J Nutr 122:1942–1951.

Holladay SD, Blaylock BL. 2002. The mouse as a model for developmental immunotoxicology. Hum Exp Toxicol 21:525–531.

Holladay SD, Lindstrom P, Blaylock BL, Comment CE, Germolec DR, Heindell JJ, et al. 1991. Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-*p*-dioxin (TCDD). Teratology 44:385–393.

Laliberté C, Dewailly É, Gingras S, Ayotte P, Weber J-P, Sauvé L, et al. 1992. Mercury contamination in fishermen of the lower north shore of the Gulf of St-Lawrence (Québec, Canada). In: Impact of Heavy Metals on the Environment (Vernet J-P ed). Amsterdam:Elsevier Science Publishers, 15–28.

Larrea E, Garcia N, Qian C, Civeira MP, Prieto J. 1996. Tumor necrosis factor alpha gene expression and the response to interferon in chronic hepatitis C. Hepatology 2:210–217.

Macdonald RW, Barrie LA, Bidleman TF, Diamond ML, Gregor DJ, Semkin RG, et al. 2000. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. Sci Total Environ 254:93–234.

Muckle G, Dewailly É, Ayotte P. 1998. Prenatal exposure of Canadian children to polychlorinated biphenyls and mercury. Can J Public Health 89:S20–S25.

Muir D, Braune B, DeMarch B, Norstrom R, Wagemann R, Lockhart L, et al. 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. Sci Total Environ 230:83–144.

Nokta M, Matzke D, Jennings M, Schlick E, Nadler PI, Pollard R. 1991. In vivo administration of tumor necrosis factor-alpha is associated with antiviral activity in human peripheral mononuclear cells. Proc Soc Exp Biol Med 197:144–149.

O'Neill LA. 2001. Dioxins damage dendritic cells. Trends Immunol 22:296.

Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 18:495–500.

Rhainds M, Levallois P, Dewailly E, Ayotte P. 1999. Lead, mercury, and organochlorine compounds levels in cord blood in Quebec, Canada. Arch Environ Health 54:40–47.

Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, et al. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334–336.

Ross PS, de Swart RL, van der Vliet H, Willemsen L, de Klerk A, van Amerongen G, et al. 1997. Impaired cellular immune response in rats exposed perinatally to Baltic Sea herring oil or 2,3,7,8-TCDD. Arch Toxicol 71:563–574.

Ryan JJ, Dewailly É, Gilman A, Laliberté C, Ayotte P, Rodrigue J. 1997. Dioxin-like compounds in fishing people from the Lower North Shore of the St. Lawrence River, Quebec, Canada. Arch Environ Health 52:309–316.

Safe SH. 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Toxicology 1:51–88.

———. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149.

Soboslay PT, Geiger SM, Drabner B, Banla M, Batchassi E, Kowu LA, et al. 1999. Prenatal immune priming in onchocerciasis-onchocerca volvulus-specific cellular responsiveness and cytokine production in newborns from infected mothers. Clin Exp Immunol 117:130–137.

Svensson BG, Hallberg T, Nilsson A, Schutz A, Hagmar L. 1994. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. Int Arch Occup Environ Health 65:351–358.

Takayama M, Itoh S, Nagasaki T, Taminizu I. 1977. A new enzymatic method for determination of serum choline containing phospholipids. Clin Chim Acta 79:93–98.

Weisglas-Kuperus N, Patandin S, Berbers GAM, Sas TCJ, Mulder PGH, Sauer PJJ, et al. 2000. Immunological effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect 108:1203–1207.

Weisglas-Kuperus N, Sas TCJ, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, et al. 1995. Immunological effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 38:404–410.

Wong GH, Krowka JF, Stites DP, Goeddel DV. 1988. *In vitro* anti-human immunodeficiency virus activities of tumor necrosis factor-alpha and interferon gamma. J Immunol 140:120–124.

Zhao Y, Dai ZP, Lv P, Gao XM. 2002. Phenotypic and functional analysis of human T lymphocytes in early second- and third-trimester fetuses. Clin Exp Immunol 129:302–308.