IMMUNOLOGICAL EFFECTS IN NEWBORNS FROM SAINT-LAWRENCE RIVER COASTAL POPULATIONS EXPOSED TO POPs AND HEAVY METALS

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Introduction

In animal models, the immunotoxic effects of POPs (mainly dioxin-like compounds) include thymus atrophy, inhibition of some components of the complement system, reduction in the blood T, B and NK cells, and immunoglobulins, inhibition of IL-1 secretion and higher levels of IL-4 and IFN-γ (1). Prenatal exposure to POPs might induce more severe and persistent effects than those induced during the postnatal exposure. In humans, massive prenatal caused a greater susceptibility to infections, a reduction in IgA and IgM levels, and a reduction in cellular immunity secondary to a reduction in T cell subsets (2). We previously reported that the risk of otitis media in Inuit infants from Northern Quebec was associated with prenatal exposure to POPs (3). Methylmercury is the major form of organic mercury and is cytotoxic for the immune system of several rodent species. In humans it can alter NK cell activity, the expression of certain T cells activation markers and it might induce allergy and autoimmune problems(4).

The Lower-North-Shore of the St. Lawrence River is located in the eastern part of Canada. Six thousands inhabitants live in 15 villages along the coastal shore. Their diet is based on species of the marine food chain, which accumulate heavy metals, lead and mercury and POPs, This population eats unusual high doses of persistent organic pollutants and heavy metals as compared to the Southern Quebec population. A study among newborns from the Quebec Lower-North-Shore region showed a reduction in naive T cells ($CD4^+$-$CD45RA^+$), a lower serum IgM level, and a slight diminution of the proliferative response of T cells following a mitogenic stimulation, as compared to newborns with low exposure to POPs and mercury (manuscript in preparation). These modifications were associated with the degree of exposure during the prenatal period, suggesting an effect on the lymphocyte functions. In the present study, we further investigated this aspect by measuring, the presence of activation markers on T cell, as well as the production of cytokines *in vitro*, following mitogenic stimulation by cord blood lymphocytes, from Lower-North-Shore newborns and newborns with lower exposure to POPs and heavy metals from nearby cities.

Methods and Materials

Population, blood sampling and processing

Women giving birth in Sept-Îles hospital were asked to participate in the study. A consent form was signed, a questionnaire was administered to document dietary and lifestyle habits and a cord

blood sample was obtained after the cord was severed. Forty-seven mothers were recruited from the Lower-North-Shore region (high exposure group);65 mothers were recruited from the Sept-Îles and Port-Cartier areas (low exposure group). Blood was collected in vacutainers with heparin as anticoagulant and kept at 20°C and sent within 48 h to the immunology laboratory. Upon reception, a 3-ml aliquot was saved for heavy metals analysis and the remaining blood was processed for lymphocyte isolation on a Ficoll-Hypaque gradient. Plasmas were stored frozen at –20°C. Whole blood and plasma samples were sent to the *Centre de Toxicologie du Québec* for the determinations of heavy metals and POPs.

Analytical chemistry analyses
A mixture of ammonium sulfate/ethanol/hexane was added to the plasma for the POPs extraction. Extracts were concentrated and purified on Florisil columns. Fourteen PCB congeners (IUPAC nos. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) and 11 chlorinated pesticides (aldrin, β-HCH, α-chlordane, γ-chlorade, *cis*-nonachlor, p,p'-DDE, p,p'-DDT, hexachlorobenzene, mirex, oxychlordane, *trans*-nonachlor) were measured by high-resolution dual-capillary column (HPUltra I/Ultra II) gas chromatography with dual Ni-63 electron capture detectors. Detection limit for most compounds was 0.02 µg/L. The concentration of total plasma lipids was estimated from total and esterified cholesterol, triglyceride phospholipid concentrations and POPs concentrations expressed on a lipid weight basis. Polyunsaturated fatty acids (PUFA) were measured in plasma phospholipids by capillary gas-liquid chromatography (B. J. Holub, University of Guelph, Ont, Canada). Blood mercury and lead were determined by atomic absorption following nitric acid digestion.

Immune system parameters
In vitro cytokine production: Lymphocytes were incubated in triplicate at a density of 2×10^5 cells/well, with PHA (1.25 µg/mL) or ConA (5 µg/mL), at 37°C during 20, 48 and 72 hours, corresponding to secretion times for the cytokines IL-2, IL-10 and TNF-α respectively. The supernatants were recovered, and cytokine levels were measured by ELISA.
Lymphocyte activation markers: CD3/CD25, CD4/CD45RO, CD8/CD45RO and CD8/DR were estimated on lymphocytes incubated at 37°C for 72 hours with ConA and stained with the appropriate FITC/PE-conjugated pairs of monoclonal antibodies and analyzed by flow cytometry.

Results and Discussion
The two groups differed with regard to ethnicity, smoking and weight before pregnancy. N-3 and N-6 PUFA levels in cord plasma phospholipids were significantly lower in the high-exposure group (Table 1). Results presented in Table 2 indicate a lower rate of IL-10 secretion by lymphocytes collected from highly-exposed newborns. No significant difference was observed for IL-2 and TNF-α. Concerning T lymphocyte activation markers on CD3+, $CD4^+$ and $CD8^+$ cells, no significant differences were noted. Plasma PCB concentration (sum of the main congeners #138,153,180) was 3.5 times higher in the high-exposure group. Differences of 1.7, 1.5 and 1.6-fold were also noted for DDE, HCB and mercury respectively. Contaminants not listed were detected in less than 50% of cord blood samples (Table 3). Correlation analyses revealed that plasma PCB concentration was negatively related to the ability of lymphocytes to produce cytokines (IL-10 and TNF-α) (Table 4).

These findings suggest that prenatal exposure to PCBs and heavy metals do not affect T-lymphocyte functions in newborns. Neither T-lymphocyte proliferation (HLA-DR expression and IL-2 secretion) nor T-cell maturation/differentiation (CD45RO) were altered. However, exposure to these xenobiotics rather seems to down-regulate some pro-inflammatory mediators such as IL-10 and TNF-α that are known to play an important role in immunological functions (B-cell proliferation/humoral responses and phagocytosis) associated with host resistance to infections. An evaluation of the susceptibility to infectious diseases in these populations is in progress.

Table 1 : Characteristics of participants

Characteristics	High exposure (n=47)	Low exposure (n=65)	P value[1]
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)	3493 (486)	3455 (479)	0.682
N-3 PUFA: mean (SD)	5.9 (1.3)	6.9 (1.4)	<0.001
N-6 PUFA : mean (SD)	28.1 (3.0)	29.2 (1.6)	0.019
[1]P value obtained by Student-t tests for means or Fisher exact tests for percentages			

Table 2 : Cytokine production and cell surface markers following stimulation of cord blood lymphocytes *in vitro* (fold induction)

	N	High exposure	N	Low exposure	P value[1]
		Geometric mean (95% CI)		Geometric mean (95% CI)	
Cytokines					
IL-2	42	39.6 (29.1-53.8)	39	36.6 (29.4-45.5)	0.675
IL-10	38	2.4 (1.9-3.1)	36	3.6 (2.7-4.8)	0.034
TNF-α	34	2.1 (1.6-2.8)	37	2.8 (2.1-3.8)	0.127
Cell surface markers					
CD3/CD25	33	16.48 (12.7-21.4)	50	18.9 (15.3-23.2)	0.420
CD4/CD45RO	33	10.6 (8.4-13.4)	49	13.5 (10.3-17.6)	0.175
CD8/CD45RO	33	19.1 (14.1-25.9)	49	30.0 (15.4-25.9)	0.820
CD8/DR	26	2.7 (1.8-4.1)	46	1.9 (1.4-2.6)	0.194
[1]P value by Student-t test on log-transformed values.					

Table 3 : Concentrations of organochlorines (μg/Kg plasma lipids) and heavy metals (nmol/L) in cord blood samples.

	% detected	Low-exposure	High-exposure	P value[1]
		Geometric mean (95%CI)	Geometric mean (95%CI)	
Polychlorinated biphenyls				
Congener 138	92.9	35.90 (26.69-48.28)	10.60 (9.33-12.05)	<0.001
Congener 153	97.3	49.69 (36.18-68.26)	14.05 (12.39-15.93)	<0.001
Congener 180	64.3	20.90 (15.37-28.41)	6.11 (5.39-6.92)	<0.001
Total PCBs (138, 153 and 180)		107.45 (79.26-145.67)	31.05 (27.52-35.04)	<0.001
Chlorinated pesticides				
DDE	100	143.76 (113.56-181.98)	83.62 (73.02-95.77)	<0.001
HCB	89.3	13.59 (11.76-15.70)	9.11 (8.21-10.10)	<0.001
Heavy metals				
Mercury	93.7	8.95 (7.28-11.00)	5.37 (4.63-6.23)	<0.001

[1]P value obtain by Student-t test on log-transformed values

Table 4 : Correlation coefficients between plasma PCB concentration and *in vitro* cytokine production.

Cytokine	Pearson's R	P value
IL-2	-0.045	0.100
IL-10	-0.191	0.037
TNF-α	-0.248	0.012

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