CONDITION OF HELPER AND SUPPRESSOR T LYMPHOCYTE SUBPOPULATIONS IN 10-MONTH-OLD JAPANESE INFANTS PERINATALLY EXPOSED TO ORGANOCHLORINE PESTICIDES, PCBs AND DIOXINS

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Introduction

Our environments including food have been polluted with some organochlorine compounds such as dioxins, polychlorinated biphenyls (PCBs) and pesticides [1, 2]. Japanese people have also been contaminated with these chemicals [3, 4]. Consequently, some pesticides such as hexachlorocyclohexans (HCHs), 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT), dieldrin and heptachlor epoxide (HCE), and PCBs have been determined in Japanese breast milk [5, 6, 7] and their mean or median concentrations on fat weight basis were about 420, 330, 3, 4 and 110 ppb, respectively [6, 7]. Their levels were considered more than 100 to 10,000 times higher than those of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs), so-called dioxins, in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) value as a whole [6]. Therefore, we should give due attention to possible health consequences of these organochlorine pesticides and PCBs as well as dioxins in Japanese infants.

We have already reported effects of the perinatal exposure to these compounds on lymphocyte subpopulations in the peripheral blood of Japanese infants [7, 8, 9, 10]. In this study, their effects on them were investigated more in detail in the same infants.

Materials and Methods

In this study, one hundred and twenty four mothers volunteered to participate in all. Pregnancy and delivery were completed without overt signs of serious illness or complications. Only babies born at term (37 to 42 weeks of gestation) without congenital anomalies or diseases were included. Breast milk (50~100 ml), sampled 2 to 4 months after childbirth, was used to determine concentrations of organochlorine pesticides and PCBs by ECD gas chromatographic method [6, 11] and dioxins by high resolution GC/MS method [6].

About 1 year after birth, 5 to 10 ml of peripheral blood samples were individually obtained from 93 infants. These blood samples were employed to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against helper (CD4 positive) and suppressor (CD8 positive) T cells, and their relative population densities were calculated [12].

In order to conduct more robust and reproducible statistical analysis, data were categorized into two groups. According to concentrations of the compounds, which were adjusted for years, and the levels of immune response variables such as percentages of the lymphocyte subpopulations and CD4/CD8 ratios, donated by 0 (less than the mean including minimum value) and 1 (the last quartile including maximum value). Then, we examined the relationship between the immune response variables and contamination levels of the organochlorine pesticides, PCBs and dioxins by simple logistic regression analysis, and calculated odds ratios. In addition, multiple logistic regression analysis was done to compute the joint effect of every two compounds, each of which showed less than 0.300 of p-value in simple logistic regression analysis, on the immune response system.

Results and Discussion

Results of chemical analyses of the organochlorine compounds in Japanese breast milk are indicated in Table 1. Concentration of ß-HCH or DDT was about 100 times higher than that of dieldrin or HCE. Contamination levels of chlordane and PCBs were around 100 ng/g lipid and 2.5 to 5 times less than those of ß-HCH and DDT. In dioxins, their TEQ levels were computed by using 1998 WHO toxic equivalency factor (TEF) values [13] and the mean concentration was 24 pg-TEQ/g lipid, which was about 200 times lower than those of dieldrin and HCE.

Table 1. Contamination levels of organochlorine pesticides, PCBs and dioxins in breast milk of Japanese mothers

Compound	Concentration	
	Mean	Standard Deviation
Organochlorine pesticide (ng/g lipid)		
ß-HCH	419	298
Dieldrin	4.3	4.4
DDT*	347	255
HCE	4.5	3.9
Chlordane**	82	60
PCBs (ng/g lipid)	136	92
Dioxins*** (pg-TEQ/g lipid)	24	9.1

* : Sum of p, p'-DDE and p, p'-DDT

** : Sum of oxychlordane, *trans*-nonaclor and *cis*-nonaclor

*** : Sum of PCDDs, PCDFs and Co-PCBs

As shown in Table 2, observed range of the percentages of helper (CD4+) T cells was larger than the normal range, which was determined for Japanese adults. Lower levels of the percentages of suppressor (CD8+) T lymphocytes were less than the lower limit and higher levels of CD4/CD8 ratios greater than the upper one.

Results of simple and multiple logistic regression analyses concerning effects of the organochlorine compounds on the immune response system are indicated in Tables 3 and 4, respectively.

In simple logistic regression analysis, HCE significantly increased the percentages of suppressor T lymphocytes in peripheral blood of Japanese infants in accordance with our previous results [10]. Effect, however, of other compounds on helper (CD4+) and suppressor (CD8+) T lymphocyte subpopulations was not observed. Dioxins only significantly enhanced CD4/CD8 ratios.

In multiple logistic regression analysis, HCE and chlordane jointly affected the percentages of CD8 positive T cells and significantly increased the odds ratio more than each of them did. We, however, do not know the clinical significance of all the results mentioned above at present.

Table 2. Percentages of helper (CD4+) and suppressor (CD8+) lymphocyte subpopulations in peripheral blood of Japanese infants

Lymphocyte Subpopulation (Positive Cells)	Percent	
	Median (min. ~ max.)	Normal Range*
CD4	39.6 (15.7 ~ 61.7)	25 ~ 56
CD8	19.1 (10.6 ~ 41.2)	17 ~ 44
CD4/CD8	2.08 (0.62 ~ 4 .52)	0.6 ~ 2.9

* : Determined by the biggest center of clinical examinations in Japan, SRL Corp., Tokyo, Japan for adults.

Table 3. Relationship between percentages of CD4 and CD8 positive lymphocytes or their ratios (CD4/CD8) in the peripheral blood of Japanese infants and perinatal exposure to organochlorine pesticides, PCBs or dioxins by simple logistic regression analysis (p-value<0.200)

Response Variable	Exposure Variable (Odds Ratio, p-value)
	Organochlorine pesticides, PCBs and Dioxins
CD4	HCE (1.54, 0.121)
CD8	**HCE (2.34, 0.002)**, Chlordane (1.44, 0.191)
CD4/CD8	**Dioxins (1.71, 0.089)**

Boldface indicates statistically significant exposure variable (p-value<0.100).

Table 4. Joint effect of two compounds perinatally exposed on percentages of CD4 and CD8 positive lymphocytes and their ratios in the peripheral blood of Japanese infants by multiple logistic regression analysis

	Exposure Variable (Organochlorine pesticides, PCBs and Dioxins)			
Response Variable	X_1	X_2	Odds Ratio	p-value
CD4	HCH (1.20)	HCE (1.48)	1.76	0.318
CD8	**HCE (2.07)**	**Chlordane (1.28)**	**2.64**	**0.016**
CD4/CD8	—	—	—	—

Number in parenthesis is odds ratio of the single compound.
Boldface shows statistically significant joint effect of the two compounds (X_1, X_2) at p-value less than 0.100.

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