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Effects of Lactational Exposure to Chlorinated Dioxins and Related Chemicals on Lymphocyte Subpopulations in Japanese Babies

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Abstract

Effects of lactational exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) on lymphocyte subpopulations were investigated in the peripheral blood of 69 breast-fed Japanese babies. As a result, estimated total intakes of PCDDs, PCDFs and Co-PCBs in toxic equivalent quantity (TEQ) converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) from the breast milk significantly and negatively correlated with the percentages of CD8-positive (suppressor/cytotoxic) lymphocytes in the blood of breast-fed babies ($p=0.043$). Consequently, the ratios of CD4-positive (helper/inducer) lymphocytes to CD8-positive lymphocytes in percentages in the peripheral blood showed increasing tendency with the estimated total TEQ intakes ($p=0.062$). Therefore, it is considered that exposure to background levels of PCDDs, PCDFs and Co-PCBs through breast milk may cause some immunologic disturbance like atopic dermatitis.

Introduction

Human beings have been contaminated with extremely toxic PCDDs, PCDFs and Co-PCBs[1)2)3)]. Consequently, these chemicals have been also determined in the human breast milk. According to their levels in the breast milk, breast-fed babies in Japan are considered to have relatively a large amount of these chemicals, namely, about 100 to 200 TEQ pg/kg body weight/ day[2)]. Babies seem more sensitive to the toxic chemicals, so we should give due attention to their possible health consequences in breast-fed babies.

In order to clarify the effects of lactational exposure to PCDDs, PCDFs and Co-PCBs on the immune system, we investigated the lymphocyte subpopulations in the peripheral blood of 69 babies in relation to their intakes from breast milk.

Experimental Methods

Eighty two mothers volunteered to participate in all in this study and they had a normal pregnancy without use of medicines. Breast milk (50~100mℓ), sampled 2 to 3 months after childbirth, was used to determine concentrations of PCDDs, PCDFs and Co-PCBs by high resolution GC-MS method and the amounts of TEQ were calculated in the breast milk using the international toxic equivalent factors (TEF) for PCDDs and PCDFs, and the TEFs by WHO-ECEH for Co-PCBs[2)3)].

About 1 year after birth, 5 to 10mℓ of peripheral blood samples were individually obtained from 69 breast-fed babies. These blood samples were used to measure lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3 (mature T-lymphocytes), CD4 (helper/inducer T-lymphocytes), CD8 (suppressor/cytotoxic T-lymphocytes), CD4 + CD8, CD16 (natural killer T-lymphocytes), CD20 (B-lymphocytes) and HLA-DR (activated T-lymphocytes), and their relative population densities were calculated[4)].

Total TEQ intakes (ng/kg body weight) were estimated by multiplying daily TEQ intakes (pg/kg body weight) of PCDDs, PCDFs and Co-PCBs as a whole from the breast milk, which were calculated with their TEQ levels in the breast milk times an expected intake of breast milk in Japanese baby, namely, 120g/kg body weight, by individual duration of breast feeding (days).

Analysis of variance (ANOVA) was applied to examine the relationship of the estimated total TEQ intakes of PCDDs, PCDFs and Co-PCBs from the breast milk to each variable of interest and statistical significance was evaluated by Student's *t*-test.

Results

1) Concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk

Respective distributions in total concentrations of PCDDs, PCDFs and Co-PCBs as TEQ on the whole and fat weight bases are indicated in Fig. 1. Median concentrations on the whole and fat weight bases were 0.95 and 25.7ppt, respectively. The range of concentrations on the whole weight basis was 0.27 to 2.53ppt and that on the fat weight basis 8.6 to 48.5ppt.

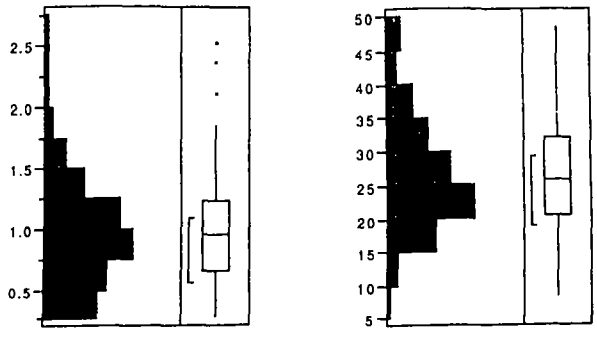

Fig. 1. Distributions in total concentrations (ppt) of PCDDs, PCDFs and Co-PCBs as TEQ on the whole (left) and fat (right) weight bases in the breast milk of 82 mothers

2) Estimated intakes of PCDDs, PCDFs and Co-PCBs from the breast milk

The distribution in estimated total intakes of PCDDs, PCDFs and Co-PCBs as TEQ in 75 breast-fed babies is shown in Fig.2. The median intake was 29.0 ng/kg and the range was 6.1 to 83.6 ng/kg.

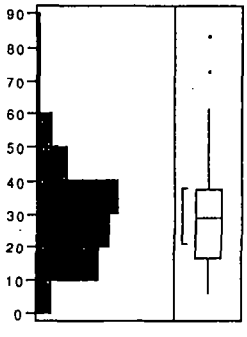

Fig. 2. Distribution in estimated total TEQ intakes (ng/kg) of PCDDs, PCDFs and Co-PCBs in 75 breast-fed babies

3) Percentages of lymphocyte subpopulations in the peripheral bloof of breast-fed babies

As shown in Table 1, median percentages of lymphocyte subpopulations in the blood of 69 breast-fed babies were as follows. Mature T-lymphocytes (CD3) was the highest, 60.4%, helper/inducer T-lymphocytes (CD4) : 39.5%, activated T-lymphocytes (HLA-DR) : 26.0%, B-lymphocytes (CD20) : 22.7%, suppressor/cytotoxic T-lymphocytes (CD8) : 19.1%, natural killer T-lymphocytes (CD16) : 8.6% and T-lymphocytes possitive to both CD4 and CD8 the lowest, 0.5%. The median ratio of CD4/CD8 was 2.05. Distributions in percentages of CD8-positive

lymphocytes and in the ratios of CD4/CD8 in percentages were shown in Fig. 3.

Table 1. Percentages of lymphocyte subpopulations in the peripheral blood of 69 breast-fed babies

Lymphocyte Subpopulation (Positive Cells)	Median (min. ~ max.) Percentage
CD3	60.4 (31.2 ~ 76.6)
CD4	39.5 (15.7 ~ 60.4)
CD8	19.1 (10.6 ~ 41.2)
CD4 + CD8	0.5 (0.2 ~ 2.1)
CD16	8.6 (1.7 ~ 23.3)
CD20	22.7 (5.5 ~ 56.2)
HLA-DR	26.0 (8.2 ~ 62.1)
CD4/CD8	2.05 (0.62 ~ 3.65)

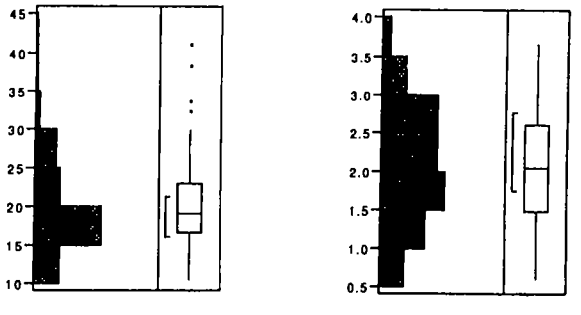

Fig. 3. Distributions in percentages of CD8-positive lymphocytes (left) and in the ratios of CD4/CD8 in percentages (right) in the peripheral blood of 69 breast-fed babies

4) Correlation between the estimated total intakes of PCDDs, PCDFs and Co-PCBs from the breast milk and the peripheral lymphocyte subpopulations in breast-fed babies.

Estimated total TEQ intakes of PCDDs, PCDFs and Co-PCBs from the breast milk significantly and negatively correlated with the percentages of CD8-positive lymphocytes in the blood of the babies ($p=0.043$). Consequently, as indicated in Fig. 4, the ratios of CD4 to CD8 in percentages in the peripheral blood showed increasing tendency with the estimated total TEQ intakes ($p=0.062$).

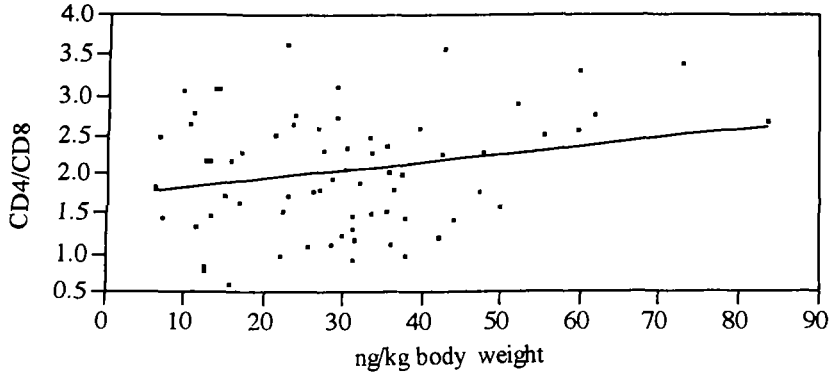

Fig. 4. Correlation between the ratios of CD4- to CD8-positive lymphocytes in percentages in the peripheral blood and the estimated total TEQ intakes in 69 breast-fed babies (p=0.062)

Discussion

The presence of PCDDs, PCDFs and Co-PCBs in the breast milk results in daily intakes of about 32 to 304 pg/kg body weight as TEQ with the median figure of 114 pg/kg body weight. Conse- quently, the babies have been estimated to take 6 to 84 TEQ ng/kg body weight with the median of 29 ng/kg body weight during whole breast-feeding periods (Fig.2).

Probably due to such kinds of relatively great TEQ intakes, we observed negatively significant correlation between the percentages of CD8-positive lymphocytes in the blood of the breast-fed babies and the estimated total TEQ intakes. Accordingly, as indicated in Fig. 4, the ratios of CD4 to CD8 seemed to proportionally increased with the estimated TEQ intakes.

Recently, no relationship between pre- and postnatal PCB/Dioxin exposure and upper or lower rispiratory tract symptoms or humoral antibody production was reported[5]. However, babies with higher CD4/CD8 ratios than 2.5 or 3.0 will be expected to become atopic dermatitis in future with high possibility (personal communication).Therefore, the results of this study suggest that exposure to background levels of PCDDs, PCDFs and Co-PCBs via breast milk may cause some immunologic disturbance such as atopic dermatitis.

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