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# Immunity of PCB Transplacental Yu-Cheng Children in Taiwan

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An epidemic of PCB poisoning began at the end of 1978 and reach its peak in March/April of 1979 in Taiwan. From dose response evaluation, incubation period were 3-4 months and consuming period persisted for about 8 months (Lan et al. 1981). The majority of affected victims were students and factory workers age 11 to 30 years. Among them there were more than 800 women of reproductive age, most of them would had been or would soon be married and pregnant after the event. Because polychlorinated biphenyls persist in adipose tissues and difficultly excrete, the offsprings of these pregnant women would be poisoned through placenta. These babies, who were poisoned only through the placenta were named as 'PCB transplacental Yusho babies' in Japan (Yoshimura 1974) and 'PCB transplacental Yu-Cheng babies' in Taiwan (Lan et al. 1987). These transplacental Yu-Cheng babies were reported to have 'fetal PCB syndrome'(FPS) (Yamashita et al. 1985; Lan et al. 1987). Bailey et al.(1980) indicated that milk concentrations about 20 times higher than maternal serum level for PCB treated rhesus monkeys. Infant serum levels of PCB were approximately two to three fold higher than serum concentrations in their mother. In general tissue levels of PCB were higher in the infants than in their mothers, and PCB were concentrated in the infant fat. As for the fetotoxicity of PCB, it may cause low viability and intrauterine growth retardation of fetuses in human being and animal model (Merson and Kirkpatrick 1976; Yen et al. 1989).

As more than 800 women of reproductive age were affected. It was expected that PCB transplacental Yu-Cheng babies would increase in this recent decade after the outbreak in 1979. Based on the age-specific fertility rates in Taiwan, it was estimated that about 270 Yu-Cheng babies would have been born to PCB-poisoned women between 1979 and 1986 from the Health Statistics in Taiwan (1986). Transplacental Yu-Cheng babies have become an important public

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health issue in Taiwan.

Up to now, there has been no studies about the immune status of PCB transplacental Yu-Cheng babies. In earlier study, Yu-Cheng babies was reported to have a high infant mortality rate (8/39=20.5%) (Hsu et al. 1985). Lan (1982) reported that these babies would easily catch cold, experience diarrhea and abdominal pains. Recently, Rogan et al.(1987) found that the bronchitis rate of Yu-Cheng babies are higher than control babies. Because of high risk of infection, it is presumed that these babies might suffer immunosuppression to certain extent. According to above findings, investigations into the immune functions of PCB transplacental Yu-Cheng babies are needed.

## MATERIALS AND METHODS

Definition of PCB transplacental Yu-Cheng children : From epidemiological data as above-mentioned we presumed the babies born after 1979 as PCB transplacental Yu-Cheng babies. These children were exposed to PCB through placenta, and possibly through breast milk, but they did not consume the PCB-contaminated oil by themselves. The clinical feature of PCB-poisoned mothers and their transplacental Yu-Cheng babies was reported by our previous study (Lan et al. 1987).

Selection of exposed and control group : Four primary schools were selected from areas of high PCB intoxication. Our exposed group was made up of transplacental Yu-Cheng children that were born to PCB-poisoned mothers after 1979. We also recruited a control group matched with cases on sex, age ( ±3 months), paternal occupation, family economic status, resident area, school and classroom. None of the mothers of control group had ever been exposed to PCB contaminated oil. Each exposed case was matched by two randomly selected controls from her/his classmates. Participation in the study was on a voluntary basis. All of the 19 exposed children (10 males and 9 females) age 7.1 to 9.0 years old (8.4 ± 0.5), were selected to participate in this study. A control group of 38 children were selected but only 32 of them (16 males and 16 females) age 7.6 to 9.0 years old (8.5 ± 0.4), agreed to participate the study. Because standard deviation of tests were so large, we used Mann-Whitney U test to determine if there was any significant difference between Yu-Cheng children and their controls.

Immunological test and hematological routine : These examinations included T-cell, suppressor T-cell, helper T-cell, active T-cell, B-cell and WBC counting, leukocytes differential counting and immunoglobulin IgG, IgA, IgM and complement C3, C4 and ANA (antinuclear antibody).

Flowcytometric study of cell surface makers. The procedures were as following. A sample of 3 ml blood was collected in EDTA tube (Becton Dickison). Mixed 50 µl blood with 50µl reagent (A,B,C,D)

reagent from Becton Dickison Simulset Immune Monitoring Test), vortexed well in low speed for 5 seconds, then incubated at 4 ° C for 20 minutes. Then 1:25 lysing solution (Coulter, whole blood quick lysing solution) was then added to lyse RBC. After 1 minute and 40 seconds, cells were washed twice with phosphates buffer saline (PH:7.4). The sample was then run with flowcytometry (Coulter flowcytometry EPICS model).

Reagents used included :
reagent A : PE and FITC labeled Anti-Leu-M3 (monocyte control)
reagent B : PE labeled Anti-Leu-4 (T cell)
FITC labeled Anti-HLA-DR (activated T cell)
PE and FITC labeled Anti-Leu-M3 (monocyte control)
reagent C : PE labeled Anti-Leu2a (suppressor/cytotoxic T cell)
FITC labeled Anti-Leu-M3 (monocyte control)
reagent D : PE labeled Anti-Leu2a (suppressor/cytotoxic T cell)
FITC labeled Anti-Leu7 (NK cell)
PE and FITC labeled Anti-LeuM3 (monocyte control)

Serological Examination. Complement C3, C4 and immunoglobulin IgG, IgM, IgA in serum were analyzed with nephelometry by using Beckman Array Protein System. ANA (Antinuclear antibody) was performed with FIAX kit (Whittaker Bioproduct) by using Hep-2 cell line (Nakamura 1974).

## RESULTS AND DISCUSSION

From Table 1, all figures were within normal range and showed no significant difference in any immunological test and hematological routine between the exposed and control group.

Previous studies of Yu-Cheng patients in Taiwan, Chang et al. (1980) reported that the serum $\alpha_2$-globulin of Yu-Cheng patients was mildly increased while the $\gamma$-globulin level was mildly decreased. These results led them to some suspicion in the suppression of humoral immunity . They suspected the reason why PCB-poisoned people got URI easily might be related to the decrease of their active T-cell. They also found the serum levels of immunoglobulin M & A in the PCB-poisoned patients decreased significantly, while the concentration of IgG was in the normal range (Chang et al. 1981). After 4 years follow-up, Lu and Wu (1985) reported that the difference in number of OKT-3 (active T-cell) and T-cell became non-significant between Yu-Cheng patients and control groups, but the number of OKT4 (helper-T cells) and OKT8 (suppressor-T cells) still had difference. These data showed that the cellular immunity in PCB-poisoned patients recovered partially.

In our study, there was no significant difference in immunity between the exposed and control group. This result is different from previous studies of Yu-Cheng patients in Taiwan. The reasons of this different result may be explained by (1) different poisoning time : subjects of our study were PCB-poisoned children since

Table 1. Comparison of Immunological test and hematological routine between exposed and control groups in 1986.

| Test | Exposed group (Mean ± SD) | Control group (Mean ± SD) |
|---|---|---|
| Total of WBC (count/$mm^3$) | 8452 ± 2183 | 8490 ± 2558 |
| Active T-cell% | 8.35 ± 3.53 | 6.36 ± 3.54 |
| T-cell % | 68.06 ± 14.17 | 64.73 ± 12.42 |
| B-cell % | 15.52 ± 6.21 | 15.35 ± 6.09 |
| T-cell subpopulation | | |
| Supressor T-cell% | 32.54 ± 5.35 | 30.92 ± 6.54 |
| Helper T-cell% | 30.80 ± 8.49 | 30.03 ± 6.98 |
| Ratio(Helper/Suppressor) | 0.99 ± 0.37 | 1.02 ± 0.32 |
| Leukocytes differential count | | |
| Leukocytes% | 39.48 ± 7.66 | 36.47 ± 9.71 |
| Monocytes% | 4.93 ± 1.81 | 4.70 ± 1.78 |
| Granulocytes% | 55.56 ± 7.27 | 58.92 ± 10.26 |
| Immunoglobulin (mg/dl) | | |
| IgG | 1123 ± 196 | 1184 ± 215 |
| IgA | 182.7 ± 72.1 | 202.8 ± 72.1 |
| IgM | 161.7 ± 70.2 | 174.7 ± 55.9 |
| Complement (mg/dl) | | |
| C3 | 126.30 ± 10.90 | 110.10 ± 16.70 |
| C4 | 20.38 ± 14.00 | 19.64 ± 10.90 |
| Antinuclear antibody | 1.14 ± 0.10 | 1.14 ± 0.14 |

*There are no significant difference in any test between two group by Mann-Whitney U test.

they were fertilized ova and the examination was carried out till 7 to 9 years after the poisoning, but the study subjects of Chang et al.(1980) was PCB-fed by themselves and the examination was carried out 1 to 2 years after the poisoning. (2) different age of study subjects : Our patients were all PCB transplacental Yu-Cheng children with age ranging from 7 to 9 years old, while the age of subjects of Chang's study ranged from 4 to 24 years old with a mean of 14.8 years old (Chang et al. 1981). (3) Because body size and cell numbers increase rapidly, so the PCB burden in these transplacental Yu-Cheng children decreased faster than adult cases. The immune status recovered naturally after the PCB level decreased profusely. (4) There were some subtle immune dysfunctions, but we have not detected especially in functional defect.

Nakanishi et al.(1985) reported that IgA and IgM of Yusho patients decreased at the early stage of disease and then returned to normal after 3-4 years. There were no significant difference in OKT-3, OKT-4, OKT-8 and OKT-4/8 between Yusho patients and

controls after 5-6 years of PCB poisoning. These results suggested when time elapsed the PCB poisoning effect will be lessened. Our Yu-Cheng babies were 7 to 9 years old, more than 6 years after the poisoning, and the immune functions of these Yu-Cheng children may return to normal, as time elapsed.

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