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Effect of Dam's Accumulated Tissue PCBs on Mouse Filial T-cell Population and T-cell Subpopulations

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Polychlorinated biphenyls(PCBs) are environmental contaminants that have been shown to alter the immune status of experimental animals (Vos and DeRoij 1972; Street and Sharma 1975; Thomas and Hinsdill 1978; Takagi et al. 1987). Accidental exposures to PCBs in humans also have been shown to affect the immune system (Shigematsu et al. 1978; Cheng et al. 1981,1982; Nakanishi et al. 1985). In those studies, however, the effects of PCBs on immune response were not evaluated in immature immune systems, such as fetuses and sucklings. The immune system is differentiating during fetal development and the developing immune system may be more sensitive to xenobiotics (Spreafico et al. 1983; Spyker et al. 1982).

In our previous reports, the transfer of PCBs by breast milk from the mouse body exceeded transplacental transfer by a factor 1000 (Takagi et al. 1986). However, the filial helper T-cell activity for IgG production was less suppressed in postnatally-exposed mice than in prenatally-exposed mice (Takagi et al. 1987). In general, antibody production is regulated by a balance of helper and suppressor T-cells, and the IgG antibody production is considerably more dependent on the helper T-cell than the IgM antibody production (Koller 1984). Therefore, the suppression of helper T-cell activity for IgG production may be derived from the decrease in the number of helper T-cells or from the increase in the number of suppressor T-cells.

The aim of the present investigation is to determine if PCBs accumulated in the dam's body alter the T-cell population and T-cell subpopulations in the thymus and spleen of filial mice.

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MATERIALS AND METHODS

Mice of inbred strain C3H/HeN, 5 weeks old, were obtained from Charles River Japan Inc. (Atsugi, Kanagawa).
Kanechlor^{R} 500, commercial PCB mixture, had an average composition of 0.09% dichlorobiphenyls, 0.21% trichlorobiphenyls, 16.47% tetrachlorobiphenyl, 51.43% pentachlorobiphenyl, 26.27% hexachlorobiphenyl, 5.22% heptachlorobiphenyl, 0.29% octachlorobiphenyl (Nakamura and Kasimoto 1977).

Dosing and treatment were previously described(Takagi et al. 1987). Briefly, PCBs were administered orally to female mice at a dose of 50 mg/kg in 0.1 ml of olive oil twice a week for three weeks with blunt-tip metal intubation needles. An additional group of female mice received olive oil alone. The elimination from tissue became retarded 2 or 3 weeks after the last dose (Matthews and Anderson 1975; Hashimoto et al. 1976; Morales et al. 1979). Four weeks after the last administration these female mice were mated with untreated males. Five to six offspring were randomly chosen from each group at 4,7,11 and 15 weeks after birth for determination of complement-dependent cytotoxic assay as described below.

Monoclonal anti-Thy-1.2 antiserum (clone F7D5) was obtained from Olac 1976 Ltd. Bichester Oxon, England. Monoclonal anti-Lyt-1.1 antiserum was obtained from Cedarlane Laboratories Ltd. Ontario, Canada. Monoclonal anti-Lyt-2.1 antiserum was obtained from New England Nuclear, Boston, Massachusetts, U.S.A. These antibodies were used at final dilution of 1:248000, 1:40, and 1:1000 in RPMI 1640 medium with 5% fetal calf serum(FCS) respectively.

Thymuses and spleens were carefully disected individually and teased apart with forceps in RPMI 1640 medium. Cell aggregates were then removed by passing through a 100-mesh stainless steel wire screen. The cell suspensions were washed three times in the same medium and resuspended in RPMI 1640 medium supplemented with 5% fetal calf serum at a concentration of 10^{7} cells/ml.

Cytotoxicity studies were performed by using a chromium(^{51}Cr) release method as follows: To 10^{7} lymphoid cells suspended in 1 ml of 5% FCS in PPMI 1640 was added, 100 µCi of sodium chromate(^{51}Cr 1 mCi/ml: New England Nuclear, Boston, Massachusetts) was added and the mixture incubated at 37°C for 45-60 min. After being washed twice, the cells were brought to a final concentration of 5 x 10^{6}cells/ml. Then 100 µl of cell suspension were incubated, in duplicate, with 100 µl of indi-

cated dilutions of antisera at room temperature for 15 min. Then 100 µl of a 1:10 dilution of low toxic rabbit complement was added, and the mixture incubated for 45 min. at 37° C. After sedimentation of the cells by centrifugation, 300 µl of the supernatant was removed and radioactivity was measured in a gamma ray spectrometer. Total releasable radioactivity was determined by freeze thawing samples containing the cells and medium. Complement control samples contained either cells, medium and complement. The percent lysis was determined by the following formula:

$$\% \text{ lysis} = \frac{\text{Experimental cpm} - \text{Complement control cpm}}{\text{Freeze-thaw cpm} - \text{Complement control cpm}} \times 100$$

and the population of Lyt-1 and Lyt-2 cells were calculated from the following formula: Lyt-1 population = (% lysis of Thy-1)-(% lysis of Lyt-2) and Lyt-2 population = (% lysis of Thy-1)-(% lysis of Lyt-1)

Individual experiments were performed at least three times. The student's t test was employed too compare differences between means.

RESULTS AND DISCUSSION

To monitor the Thy-1 populations in the thymus and spleen, the complement-dependent cytotoxic assay was done with monoclonal anti-Thy-1.2 antibody at 4,7,11 and 15 weeks after birth. Fig.1 summarizes the percent of Thy-1 population in thymus and spleen compared to control mice of the same age. These data show that relative populations of all expressing Thy-1 antigens in thymus and spleen of PCBs-exposed filial mice are similar to those of the control mice throughout the experimental period.

To monitor the Lyt-1 populations in thymus and spleen, the complement dependent cytotoxic assay was carried out with monoclonal anti-Thy-1.2 antibody and monoclonal anti-Lyt-2.1 antibody at the same weeks as described before. Fig.2and3 summarize the percent of Lyt-1 populations in thymus and spleen of the PCBs exposed filial mice compared to control mice of the same age. At 4 weeks of age, the Lyt-1 populations were depressed to about 80% of control in the thymus and 60% of control in the spleen. At 7 weeks of age, they remained at about 60% of the controls in the thymus and spleen. After 11 weeks of age, however, the Lyt-1 populations increased to about 120-130% of the control in both organs.

To monitor the Lyt-2 population in thymus and spleen, the

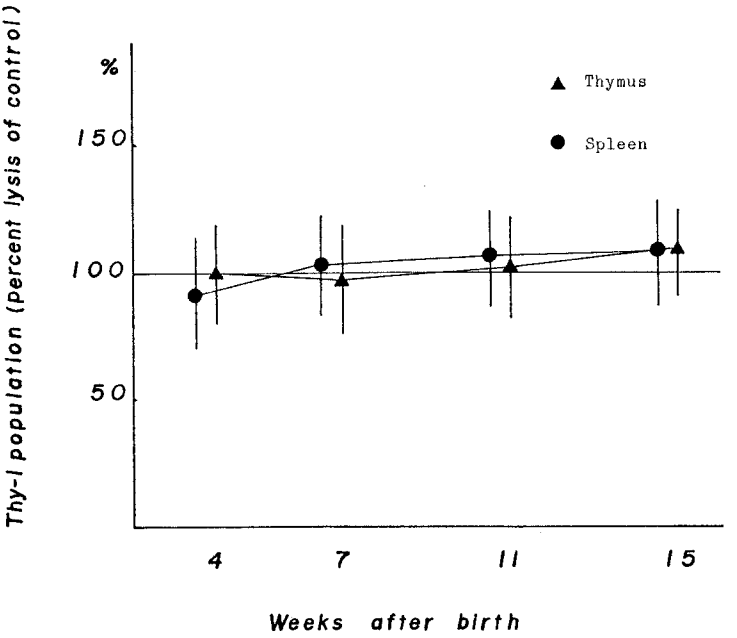

Figure 1. Affect of dam's accumulated tissue PCBs on filial Thy-1 population in thymus and spleen. Each point and its vertical line represent mean±S.D. (n=5or6)

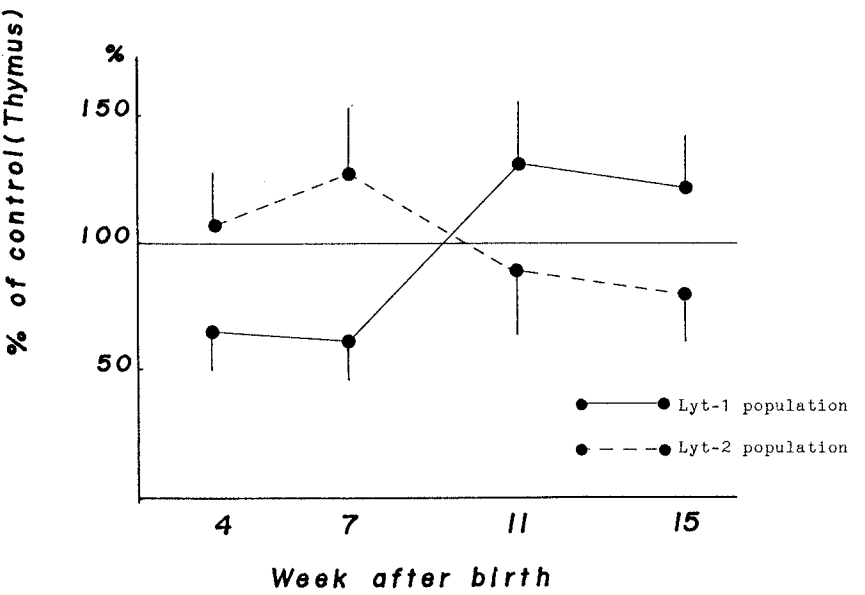

Figure 2. Affect of dam's accumulated tissue PCBs on filial Lyt-1 and Lyt-2 populations in thymus. Each point and its vertical line represent mean±S.D. (n=5or6)

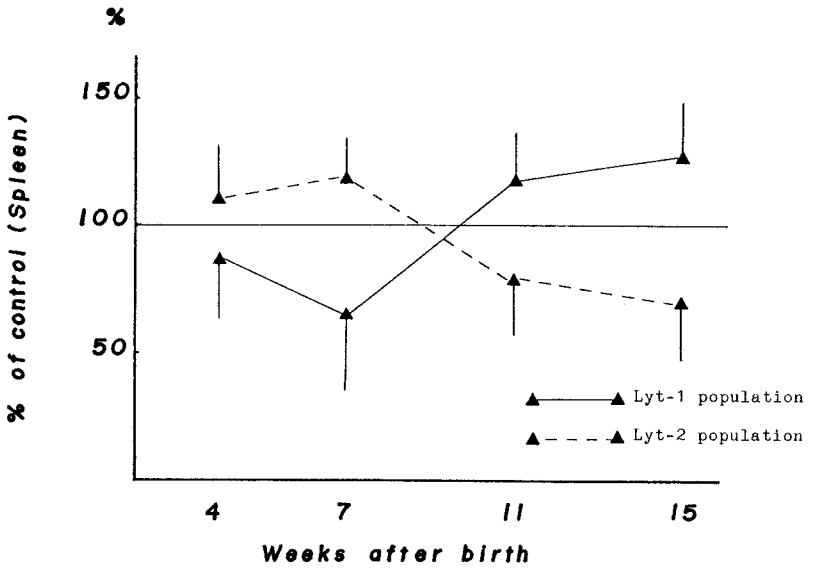

Figure 3. Affect of dam's accumulated tissue PCBs on filial Lyt-1 and Lyt-2 populations in spleen. Each point and its vertical line represent mean±S.D. (n=5or6)

complement-dependent cytotoxic assay was done with monoclonal anti-Thy-1.2 and monoclonal anti-Lyt-1.1 antibody at the same weeks as described before. Fig.2 and 3 summarize the Lyt-2 population in thymus and spleen of the PCBs-exposed filial mice compared to control mice of the same age. At 4 and 7 weeks after birth, the Lyt-2 population increased to about 110-130% of control in both organs. After 11 weeks of age, however, these population gradually decreased to about 90-70% of the controls.

Accumulation of PCBs in the dam's body have been shown to decrease the mouse filial helper T-cell activity of IgG productions(Takagi et al. 1987). Since IgG production is regulated by a balance of helper and suppressor T-cells,this study was undertaken to evolute the effects of maternally accumulated PCBs on filial T-cell populations and T-cell subpopulations. Many studies describe the presence of Thy-1 antigen on T-cell surface, and the Thy-1 antigen has been commonly used as a marker to distinguish T-cells. Furthermore, there does not appear to be any differential distribution of Thy-1 antigen on functional T-cell subsets(McKenzie and Potter 1979). In the PCBs-exposed filial mice, the populations of cell expressing Thy-1 antigen in thymus and spleen are similar to those of the control mice throughout the experimental period(Fig. 1). These

results suggest that maternally accumulated PCBs do not affect the T-cell population of their offspring.

Among T-cells, the three subsets can be characterized with Lyt-1.2.3, Lyt-1 and Lyt-2.3. The Lyt-1 subset have been identified as the phenotype of helper T-cell and the Lyt-2.3 subset have been identified as that of the suppressor T-cell for antibody production and also of the killer T-cell(McKenzie and Potter 1979). In this report, the Lyt-1 populations in PCBs-exposed filial mice were reduced to about 60-80% of control mice in both thymus and spleen at 4 and 7 weeks after birth(Fig.2 and 3). On the other hand, the Lyt-2 populations increased to about 110-130% of control mice in both organs at the same weeks of age(Fig.2 and 3).

After the 11 weeks of age, however, the Lyt-1 populations in PCBs-exposed filial mice increased to 120-130% of control mice in these two organs, while the Lyt-2 populations were decreased to about 70-90% of control mice(Fig.2 and 3). These results suggest that PCBs accumulated in the dam's body decrease the number of helper T-cells and increase the number of suppressor T-cells at 4 and 7 weeks after birth and vice versa after 11 weeks of age.

In our previous report, the helper T-cell activity in IgG production was decreased at 4 and 7 weeks after birth in the filial mice which had been prenatally exposed to PCBs, and these suppression show a gradual recovery to the control level (Takagi et al. 1987). These suppressions in IgG production in the previous report must have been derived from a decrease in helper T-cell number and an increase in suppressor T-cell number at 4 and 7 weeks after birth, and the recovery of this suppressed helper T-cell activity might have been derived from an increase in helper T-cell number and a decrease in suppressor T-cell number after 11 weeks of age.

In the case of accidental PCBs exposure in humans, the percentage of total T-cells, active T-cells and Tu-cells (helper T-cells) were decreased, while Tr-cells(suppressor T-cells) were not affected in the peripheral lymphocytes at one or two years after the exposure(Chang et al. 1981). On the other hand, at 14 years after exposure to PCBs, helper T-cells were increased and suppressor T-cells were decreased in peripheral lymphocytes (Nakanishi et al. 1985). These T-cell subpopulation changes in patients by PCBs-poisoned are in close agreement with the results in this report. Therefore,PCBs decrease the number of helper T-cells and increase or do not affect the number of suppressor T-cells in the early stage, while in the

later stage, PCBs increase the number of helper T-cells and decrease that of suppressor T-cells.

In this report, however, the use of cytotoxicity has meant that T-cell subpopulations cannot be studied directly, since treatment with either anti-Lyt-1 or anti-Lyt-2 antisera removes their T-cell subpopulations. Further studies should be carried out with some other separation methods which are not based on cytotoxicity (e.g. flow cytometry with fluoresence activated cell sorter) in order to understand the whole picture of T-cell subsets altered by maternal PCBs.

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