CHANGES IN THYMOCYTE DEVELOPMENT AND THYMIC EMIGRANTS IN RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

Keiko Nohara[1,3], Hidekazu Fujimaki[1,3], Hiroko Ushio[1], Takahiro Kobayashi[1,3], Shin-ichi Tsukumo[1,3], Mika Kijima[1,3], Yuichi Miyabara[1,3], Junzo Yonemoto[2,3] and Chiharu Tohyama[1,3]

[1] Environmental Health Sciences Division and [2] Regional Environmental Division, National Institute for Environmental Studies, Onogawa, Tsukuba, Ibaraki 305-0053, Japan
[3] CREST, JST (Japan Science and Technology), Kawaguchi, Saitama, 332-0012, Japan

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly persistent environmental pollutant and shown to affect immune functions (1). One of the primary targets of TCDD is thymus, the organ in which T lineage lymphocytes proliferate and differentiate. *In vivo* exposure to TCDD reduces thymic cellularity, mainly by decreasing the number of $CD4^+CD8^+$ double positive immature thymocytes, the most abundant cell population in thymus (2-4). In addition, skewing of thymocyte differentiation towards $CD8^+$ single positive T cells occurs in animals perinatally exposed to TCDD, and more obviously in fetal thymus organ cultures in the presence of TCDD (5,6). The subset skewing towards $CD8^+$ T cells is also reported in baby mice exposed to another Ah receptor-binding xenobiotics, 3,3',4,4'-tetrachlorobiphenyl (TCB) (7). However, direct evidence showing the relevance of TCDD-induced developmental alteration in thymocytes to physiological or functional changes in T cell pools in the whole body has been hardly obtained. It has not been clear whether the TCDD-elicited reduced cellularity in thymus, where more than 95 % of double positive cells are originally destined to be eliminated by apoptosis in the course of normal thymocyte maturation, has any significant effect on the overall pool of T cells. In rats, but not in mice, majority of recent thymic emigrants was shown to be defined by $Thy1^+CD45RC^-$ phenotype (8). In the present study, thymocytes and lymph node T cells were investigated in rats administered TCDD with a single oral dose of 1 or 2 μg/kg body weight using monoclonal antibodies against lymphocyte surface markers, in order to seek the effect of TCDD-exposure on T cell pool.

Materials and Methods

In vivo treatment with TCDD: Female SD rats (5 weeks old) were supplied by Clea Japan (Tokyo) and allowed to acclimate for one week prior to use in experiments. Then, 5 animals per group were administered TCDD in corn oil with a single oral dose of 1 or 2 □g/kg body weight or vehicle alone. On day 7, they were sacrificed and their thymi and mesenteric lymph nodes were removed immediately.

Cell preparation and flow cytometry: Single cell suspensions of thymi and lymph nodes were prepared by expressing cells in RPMI1640 medium-10 % FCS through a stainless steel mesh. Cell numbers were counted with a hemocytometer. Cells were stained with monoclonal antibodies against lymphocyte surface markers for 30 min on ice. After washing, the cells were treated with 7-aminoactinomycin D (Sigma) to label dead cells and measured using a FACSCalibur (Becton Dickinson). Live cells were gated and analyzed as described previously (9). The following monoclonal antibodies against rat surface markers were used: anti CD4-PE, anti CD8-FITC, anti Thy1.1-PE, anti CD45RC-biotin (Pharmingen) and streptavidin-FITC (Serotec).

Results and Discussion

When SD rats were orally administered 1 or 2 □g TCDD/kg and examined on day 7, the average thymus weight and thymocyte numbers were smaller than those in the control group, although the differences were not statistically significant (Table I). The ratio of $CD4^+$ single positive cells/$CD8^+$ single positive cells in thymocytes was significantly smaller in the TCDD-treated groups compared to the control group (Table II). These shifts in thymus weight and thymocyte population agreed with the results obtained in previous studies *in vivo* and fetal thymus organ cultures (5-7).

In those rats, the ratio of $Thy1^+CD45RC^-$ recent thymic emigrants in mesenteric lymph node cells was shown to significantly decrease in animals exposed to 1 or 2 □g TCDD/kg as shown in Table III. The ratio of $CD3^+$ T cells in mesenteric lymph nodes was also reduced dose-dependently by TCDD exposure albeit not significant. While the ratio of $CD8^+$ single positive cells in thymi increased in TCDD-exposed animals as described above, the ratio of $CD8^+$ cells in the lymph nodes was significantly reduced in rats exposed to 1 □g TCDD/kg (6.67□ 0.67 % versus 9.96 □0.81 % in control, P□ 0.03). Changes in the ratio of $CD4^+$ cells in the lymph nodes from control and TCDD-exposed rats were not significant. A similar result has been reported by Smialowicz et al (10) that $CD8^+$ splenocytes were reduced in a dose-dependent manner in rats given a single intraperitoneal injection of TCDD at 3, 10, or 30 □g/kg. These facts may imply that the $CD8^+$ cells generated in the TCDD-exposed thymi are less potent in proliferation than those maturated in control thymi.

All these results suggested that TCDD-administration affects the immune system by modulating thymocyte development and reducing the supply of T cells from thymus to lymph nodes, even at the doses which slightly reduce the thymocyte number. The consequent effects of reduction in the ratio of T cells, especially of $CD8^+$ T cells, in lymph nodes are now open to be studied.

Table I. Effects of TCDD exposure on thymus weights and Thymus cell numbers

Dose (□g/kg)	Relative weight (mg/g body weight)	Cell number (x 10^8)
0	2.60 ±0.22 [a)]	16.80 ± 1.81
1	2.43 ±0.12	15.19 ±1.79
2	2.25 ±0.19	14.96 ±2.10

a) Mean ±SE

Table II. Effects of TCDD on differentiation of single positive cells in thymus

Dose (□g/kg)	Ratio of CD4 SP/CD8 SP
0	3.32 ±0.45
1	2.28 ±0.08 *
2	2.09 ±0.17 *

* P□ 0.05 versus vehicle control.

Table III. Reduction of recent thymic emigrants in lymph node cells by TCDD exposure

Dose (□g/kg)	Recent thymic emigrants in lymph node cells (%)
0	26.9 ± 1.31
1	21.4 ± 0.70 ***
2	21.7 ± 1.14 **

** P□ 0.03, *** P□ 0.01, versus vehicle control.

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