EFFECT OF A SINGLE ORAL DOSE OF 2,3,7,8-TETRACHLORO-DIBENZO-*p*-DIOXIN ON IMMUNE FUNCTION IN NC/Nga MICE

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Introduction

Incidence of allergic diseases such as asthma and atopic dermatitis has been reported to increase in developed countries in recent years, and it has been suspected that environmental factors might have been responsible at least in part for the increase of the allergic inflammation. Among the environmental factors, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) would be one of the plausible candidates since exposure to TCDD induced immunosuppressive effects on resistance against infection and delayed type hypersensitivity reactions [1-3]. Since cell-mediated immunity such as susceptibility to infection, cytotoxic T cell function and delayed type hypersensitivity is regulated by Th1 type helper T cells, but not by Th2 type, TCDD may modulate the balance of immune response. Recently, NC/Nga mice have been reported to show atopic dermatitis-like lesions in accordance with an increase of plasma IgE when breeding under conventional condition [4,5]. Infiltration of $CD4^+$ T cells and macrophages was seen in the skin lesions. It is suggested that NC/Nga mice is a very useful model for investigating the causal relationship of environmental and genetic factors in induction of atopic dermatitis. We hypothesized that environmental chemicals affect the imbalance of Th1/Th2, resulting in an increase of allergic inflammation. In this study, to investigate the ability of TCDD to induce atopic-dermatitis like features in NC/Nga mice, the mice were administered with a single dose of TCDD.

Materials and Methods

Animals

Male NC/Nga mice (5-week-old) were purchased from SLC (Tokyo, Japan). After one week quarantine period, the mice were used for the experiment.

Exposure to TCDD

Each group of mice was administered TCDD in corn oil with a single oral dose of 0 (vehicle alone), 5 or 20 É g/kg body weight. On day 28, mice were sacrificed under ether anesthesia and thymus, spleen, mesenteric lymph node (MLN), ear, skin and blood were collected.

Cell preparation and flow cytometry

Cell suspensions of each tissue that had been filtered through a stainless steel mesh in RPMI-1640 medium (Dainippon Pharmaceutical Co., Osaka), containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine (Gibco RBL, Life Technologies, NY), 0.1 mM nonessential amino acids (Gibco RBL), 5 mM HEPES (Nacalai Tesque, Kyoto), penicillin (100 units/ml) and streptomycin (100 É g/ml) (ICN Biomedicals, Aurora, OH), pH 7.2-7.3 were centrifuged, counted and stained. The immunophenotype of the cells from each group of mice was analyzed by using flow cytometry. The mAbs used, purchased from Pharmingen (San Diego, CA), were as follows: phycoerythrin (PE)-labeled rat IgG anti-CD4 (clone GK1.5), FITC-labeled rat IgG anti-

CD8a (clone 53-6.7), hamster IgG anti-CD3 PE (clone 145-2C11), rat IgG anti-CD45R/B220 FITC(clone RA3-6B2), and the corresponding isotype-matched controls. Cell samples were

analyzed on a FACSCalibur flow cytometry (Becton Dickinson Immunocytometry Systems, Mansfield, MA).

Assay for total IgE antibody titer in plasma

Total IgE antibody titers in plasma were measured by using ELISA. Briefly, 96-well microimmunoplates (Nunc) were treated overnight with anti-mouse IgE monoclonal antibody, blocked by Block Ace (Dainippon Pharmaceutical Co.) and incubated with 100 É l aliquots of the diluted samples or standards for 1 h at 37 Åé. After six washings, 100 É l biotinylated anti-mouse IgE (Pharmingen) diluted 1/1000 was added to each well for 30 min at 37 Åé. After washing, each well was incubated with 100 É l streptavidin-horseradish peroxidase conjugate (Gibco) for 1 h at room temperature. After an additional wash, the substrate solution was added and developed. The OD at 490 and 595 nm was determined with a microplate reader (Model 550; Bio-Rad).

Histochemical analysis

Ear and skin tissues collected from the back were fixed with 10% buffered formaline solution. Each section was stained with hematoxylin and eosin.

Results and Discussion

A single oral administration of 20 É g TCDD/kg increased both the wet weight of mesenteric lymph node (MLN) and the ratio of wet weight/body weight of MLN and spleen (Table 1). The numbers of MLN and spleen cells were not significantly increased. Five É g TCDD /kg administration had no effect on the weights of MLN and spleen. No alteration was observed in thymus weight and its cellularity of the TCDD-administered mice.

The percentage of $B220^+$ B cells in spleen from mice exposed to 20 É g TCDD/kg was significantly increased, but that of $CD3^+$ T cells and $CD4^+$ T cells in spleen and MLN was decreased.

TCDD administration at a dose of 5 and 20 É g TCDD/kg significantly decreased total IgE titers, in plasma compared with that of control mice (Fig.1).

In all experimental periods, histochemical observation on ear and skin on the back showed no significant difference between TCDD-exposed and control mice.

TCDD administration induced the suppression of IgM antibody response to SRBC in mice [6,7] and abrogated the production of IgG2a in mice [8]. On the other hand, treatment with TCDD increased unstimulated antibody production from murine spleen cells [9] and increased IgE production from B cells that were stimulated with IL-4 and CD40 [10]. It is a controversial matter how TCDD affects antibody production. These differences may be due to the mouse strain, timing of TCDD administration with antigenic stimulation *in vivo* and *in vitro* and the difference of immunoglobulin isotypes.

Our findings suggest that exposure of NC/Nga mice to TCDD modulates innate immune functions and suppresses IgE antibody production in relation to induction of allergic diseases.

In conclusion, TCDD administration in NC/Nga mice induced the changes in cellularity of spleen and MLN, but not thymus. Moreover, plasma IgE levels markedly decreased by TCDD exposure. Since other studies showed a decreased DTH response in offspring after perinatal TCDD exposure, further studies are necessary to demonstrate the effects on allergic response in offspring of NC/Nga mouse dams orally dosed with TCDD.

Table 1. Effects of TCDD on organ weights in NC/Nga mice

TCDD (É g/kg)	Body wt (g)	Spleen (Sp/Bw)# (mg)	MLN (MLN/Bw)#	Thymus (Thy/Bw)#
0	27.5Å}1.0	63Å}3 (2.28Å}0.07)	18Å}2 (0.65Å}0.04)	37Å}2 (1.35Å}0.08)
5	25.6Å}1.2	67Å}4 (2.54Å}0.21)	20Å}1 (0.79Å}0.05)	36Å}4 (1.39Å}0.14)
20	26.7Å}0.2	67Å}2 (2.52Å}0.06*)	23Å}1* (0.86Å}0.03**)	37Å}2 (1.38 }0.07)

#Organ wt (mg)/body wt (g). * and ** denote a statistical significance from vehicle treated control at P< 0.05 and P< 0.01, respectively, by ANOVA.

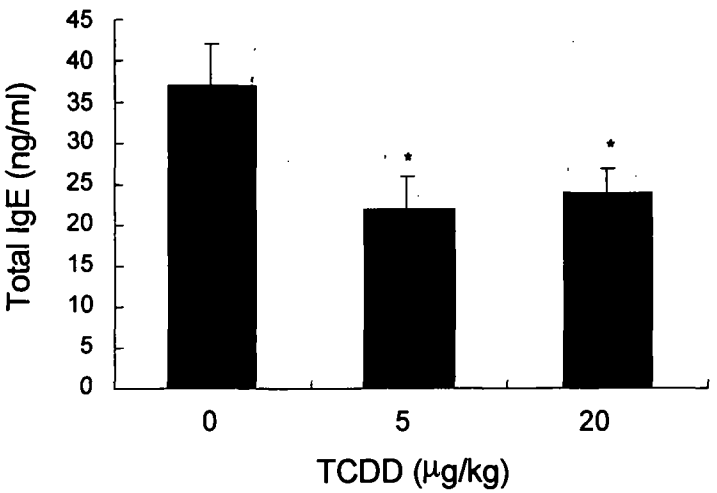

Fig. 1. Suppression of IgE antibody production in TCDD-exposed NC/Nga mice

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