OBSERVATION OF ANTIGEN-SPECIFIC IMMUNOGLOBULIN PRODUCTION IN 70-DAYS LONG-TERM TCDD-EXPOSED MICE SENSITIZED WITHOUT ADJUVANTS

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other polyhalogenated hydrocarbons, including biphenyls and furans, are generated from waste incinerator as undesired by-products. As a result, such compounds have found throughout the environment, and have shown adverse effect for human health. We usually intake such compounds via food, air water etc., especially, human intake approximately 90% of total exposure via food [1)]. However, human has biological defense mechanism for disease prevention. For example, the intestinal tract represents the first barrier to ingested environmental pollutant such as TCDD. In the intestine, numerous populations of T and B lymphocytes, dendritic cells (DCs), macrophages, granulocytes form a mucosal network known as the common-mucosal immune system (CMIS) [2)]. The CMIS links inductive and effector tissues and also play a key role in the induction of antigen-specific immune responses. The CMIS inductive site for orally administered antigen is the gut-associated lymphoid tissue (GALT), such as Peyer's patch (PP). Immunotoxicological studies in mice exposed to TCDD showed drastic changes of many immune cells, including changes in cytokine profiles [3, 4)]. The correlated systemic effects are strong innumosupression of the humoral, cellular, and innate immune response [5, 6)].

Though the major route of uptake is via food, little is known until now on the immunotoxic effects of TCDD on the GALT. And, almost all studies used adjuvant such as alum, and expose to TCDD is acute or subchronic. In this paper, we evaluated the immunotoxic effects of long-term (70 days) expose to TCDD on mice sensitized with OVA without adjuvants such as alum.

Material and methods

1) *Animals*

Female BALB/c mice (6 weeks old) were purchased from SLC, Inc. The mice were housed at 23 ± 1.5 ˚C with a 12-h light/dark cycle and were allowed free access to standard rodent chow and water. After their arrival, the mice were allowed to adapt to their environment for at least 1 week before the experiments. The animal experiments were performed according to the guidelines of Setsunan University.

2) *Chemicals*

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was dissolved in saline containing 10% Tween20 and 1% ethanol.

3) *Oral immunization and sample collection*

Mice were orally immunized with 100-µl aliquots of ovalbumin (OVA) or a mixture of OVA and TCDD at ever day for 70 days. On days 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70, serum and mucosal secretion (fecal extracts) were collected. Fecal pellets (100 mg) were suspended in 1 ml of PBS and extracted by vortexing for 10 min. The samples were centrifuged at 3,000g for 10 min, and the resultant supermatants were used as fecal extracts.

4) *OVA-specific antibody production by enzyme-linked immunosorbent assay (ELISA)*

The titers of OVA-specific antibody in serum and fecal extracts were determined by ELISA. Briefly, an immunoplate was coated with OVA (100 µg/well in a 96-well plate). Ten-fold serial dilutions of these samples were added to the immunoplate followed by the addition of horseradish peroxidase-conjugated anti-mouse IgG or IgA. The OVA-specific antibodies were detected using TMB peroxide substrate. End-point titers were expressed as the dilution ratio, which gave 0.1 above control values obtained for serum of naïve mice at an absorbance of 450 nm.

Result and discussion

To examine whether the TCDD have the confusion of immune response, especially production of OVA-specific immunoglobulin on OVA-sensitized mice without adjuvants, we orally administrated a mixture of OVA, a famous model antigen, and TCDD at every day for 70 days. Firstly, to determine the dose of TCDD with no systemic wearing disease, we monitored body weight for 70 days. On Day 70, TCDD exposure at 5, 50, and 500 ng/kg had no effect on the body weight of the vehicle-gavaged controls (Table 1). However, all mice of 1000 ng TCDD/kg died on Day 14. We found that the dose of long-term TCDD expose is lower than 500 ng TCDD/kg.

Table 1 Effects of TCDD on body weight of OVA-sensitized mice.

TCDD (ng/kg)	Body weight (g)
0	21.3 ± 0.32
5	21.8 ± 0.11
50	21.3 ± 0.25
500	21.5 ± 0.41

Mice were orally administered OVA and TCDD for 70 days, and examined on Day 70. Values represent the mean ± SEM (n=5).

Mucosal tissues such as intestine contain immunocompetent cells for adaptive immunity. B and T lymphocytes form a dynamic mucosal network for the induction and regulation of secretory IgA and cytotoxic T lymphocyte responses. As shown in Fig.1, the antibody titers of serum OVA-specific IgG and fecal OVA-specific IgA on Day 70 were increased in a dose-dependent manner. Serum OVA-specific IgG titers of 5, 50, or 500 ng TCDD/kg were increased 78.8, 220, or 38.2-fold of vehicle-administrated mice, respectively. Similar results were observed in fecal OVA-specific IgA titers. Fecal OVA-specific-IgA titers at 5, 50, or 500 ng TCDD/kg are 2.3, 3.3, or 0.4-fold of vehicle-administrated mice, respectively. Interestingly, both OVA-specific IgG and IgA titers of 500 ng TCDD/kg were decreased rather than those of 5 and 50 ng TCDD/kg. This reason is presently unclear, but the difference in dose of TCDD might show adjuvanticity or immunosuppression effects.

Further study is needed to clarify their mechanism of immune response confusion by TCDD on OVA-sensitized mice without adjuvants.

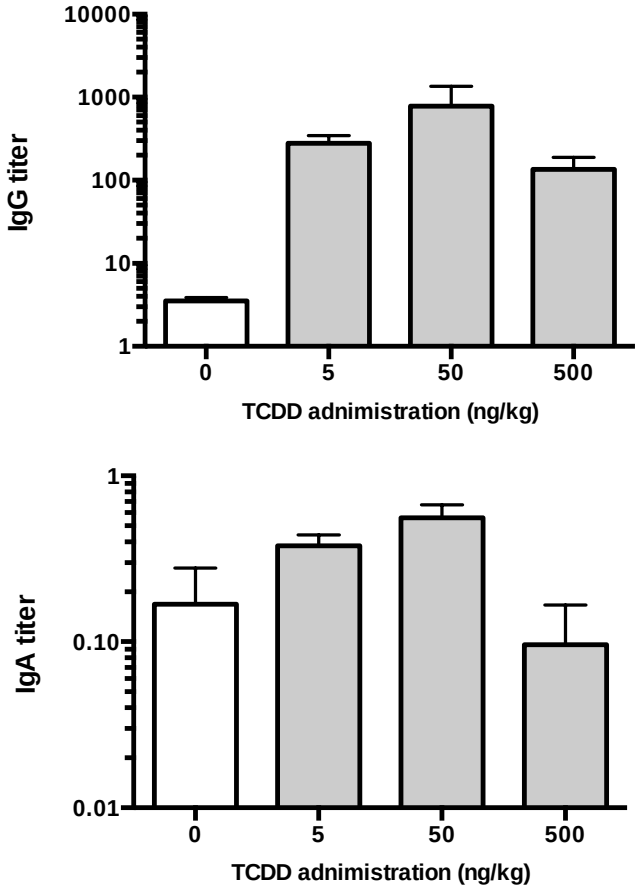

Fig. 1 Production of OVA-specific IgG and IgA in mice exposed with TCDD. Mice were orally immunized with OVA and TCDD. On Day 70, the levels of serum IgG and fecal IgA were determined by ELISA. Values represent the mean ± SEM (n=5).

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