TCDD FACILITATES THE ANTIGEN-SPECIFIC IMMUNOGLOBULIN PRODUCTION UNDER ORAL OR NASAL ADMINISTRATION

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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) (Fig. 1).

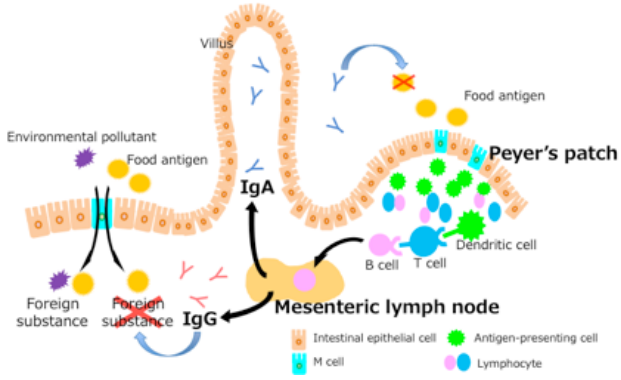

Fig. 1 Intestinal mucosa defense mechanism against harmful substance.

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 the other hand, the association between the increase in the amount of scattering of pollen, yellow sand and PM2.5 and the onset and exacerbation of allergic diseases are regarded as profound problems. It was reported that rat basophil cells stimulated with the extract of yellow sand upregulated the cytokine production, and preexposure of PM2.5 were shown the increment of inflammatory cells, interleukin-6 (IL-6) and tumeor necrosis factor-α (TNF-α) [5, 6]. Thus, it was also consided important to immune response by nasal exposure of environmental pollutants. The nasal immune play a nasopharynx-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT).

However, these reports only affect the immunocompetent cells, and the influence on the actual immune system remains unknown. In this study, we evaluated the effects of TCDD, as model compounds of environmental pollutants, on the GALT and NALT, focusing on antigen-specific immunoglobulin production.

Materials 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 or nasaly immunized with ovalbumin (OVA) or a mixture of OVA and TCDD at ever day. On every weeks, 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.

Results 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.
After 21 days, osteocalcin (left) and RUNX2 (right) mRNA level were measured by realtime-PCR. Mice were orally administered OVA and TCDD for 70 days, and examined on Day 70. Values represent the mean ± SEM (n=5).

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

Mucosal tissues such as intestine and nasal 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. Fig. 2A shows OVA-specific IgG titers on oral TCDD-exposued mice. From the 5th week after the administration, an increase in the anti-OVA IgG titer depending on the dose of TCDD was observed. The antibody titers of serum OVA-specific IgG on Week 10 were increased in a dose-dependent manner. Serum OVA-specific IgG titers of 0.5, 5, 50 or 500 ng TCDD/kg were increased 8.6, 15.5, 30.1 or 6.9-fold of vehicle-administrated mice, respectively. On the oher hand, from the 2nd week after the administration, an increase in the anti-OVA IgG titer depending on the dose of TCDD was observed on nasal exposed mice (Fig. 2B). Compared to the vehicle group, it was confirmed that anti-OVA IgG titers tended to be higher as the concentration of OVA/TCDD mixed solution at low concentration.
Similar results were observed in fecal OVA-specific IgA titers (Fig. 3). And, oral exposure of TCDD was a more sensitive immune response than nasaly exposure. Interestingly, for both oral and nasal exposure, TCDD increased antigen-specific antibody titer in a dose-dependent manner, but a decrease in antibody titer was observed at a certain dose level. This reason is presently unclear, but the difference in dose of TCDD might show adjuvanticity or immunosuppression effects.

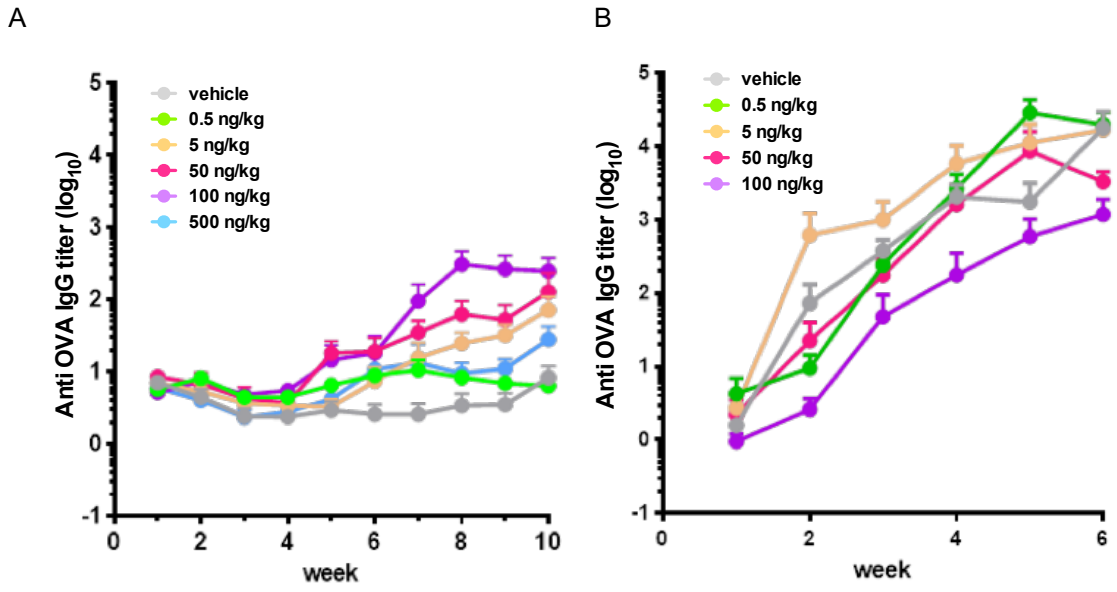

Fig. 2 Production of OVA-specific IgG in mice exposed with TCDD.
Mice were orally (A) or nasaly (B) immunized with OVA and TCDD. On every week, the levels of serum IgG were determined by ELISA. Values represent the mean ± SEM (n=5).

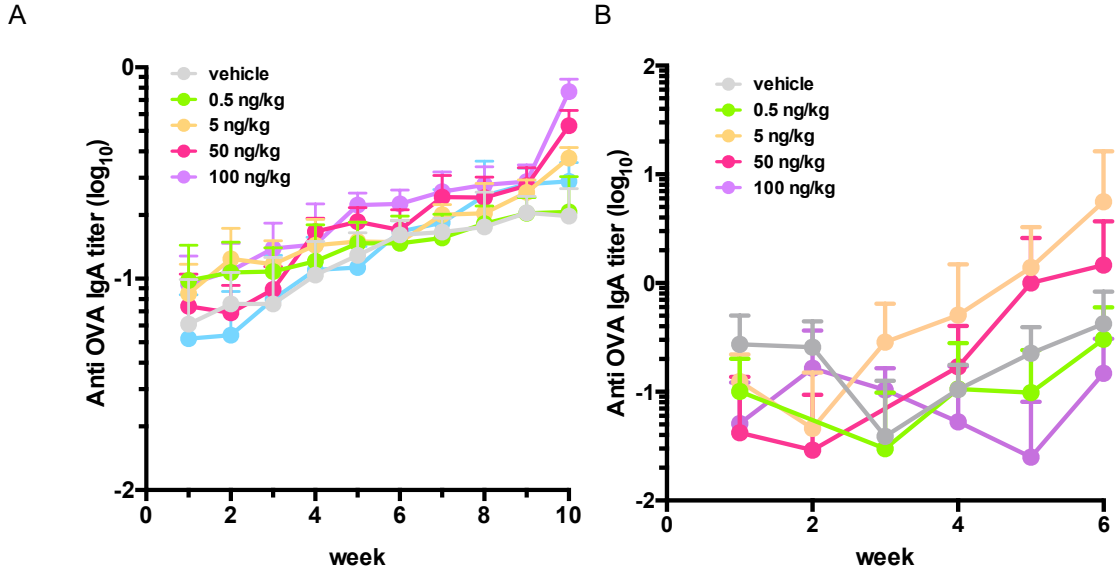

Fig. 3 Production of OVA-specific IgA in mice exposed with TCDD.
Mice were orally (A) or nasaly (B) immunized with OVA and TCDD. On every week, the levels of serum IgA were determined by ELISA. Values represent the mean ± SEM (n=5).

In order to examine the Th1/Th2 balance due to TCDD exposure, antibody titers of IgG1 (Th2-based) and IgG2a (Th1-based), which are subclasses of IgG antibody, were measured. As a result, IgG1 titers were higher than those of IgG2a titers in each group for both oral and intranasal administration. This suggests that chronic oral and nasal exposure to TCDD may shift the Th1/Th2 balance to Th2 and enhance the allergic reaction.

In conclusion, we showed that oraly and nasaly TCDD exposure were increased immne response such as antigen-specific immunogrobulin production. Further study is needed to clarify their mechanism of immune response confusion by TCDD on OVA-sensitized mice without adjuvants.

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