# Effect of oral or nasal exposure of TCDD on antigen-specific immunoglobulin production without adjuvants

Tomohiro Yuzuriha*, Hideki Kakutani, Teruyuki Nakao, and Souichi Ohta

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka, 573-0101, Japan

## Introduction

We usually unintentionally intake environmental pollutants via food, air water etc., especially, human intake approximately 90% of total exposure via food [1]. As a result, such compounds have shown adverse effect for human health. However, human has biological defense mechanism for disease prevention and such compounds. For example, the intestinal tract represents the first barrier to ingested environmental pollutant. In the intestine, numerous populations of T and B lymphocytes, dendritic cells, macrophages, granulocytes form a mucosal network known as the common-mucosal immune system (CMIS). 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). It was known that several environmental pollutants disorder the CMIS network. For example, oral exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was drastic changes of many immune cells, including changes in cytokine profiles [3,4].

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 tumor necrosis factor-α (TNF-α) [5, 6]. Thus, it was also considered 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.

**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 or nasally immunized with ovalbumin (OVA) or a mixture of OVA and TCDD at every 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 3,000*g* for 10 min, and the resultant supernatants 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 or nasally administrated a mixture of OVA, a famous model antigen, and TCDD at every day for 10 or 6 weeks, respectively. Firstly, to determine the dose of TCDD with no systemic wearing disease, we monitored body weight for 10 or 6 weeks. On Week 10 or Week 6, TCDD exposure had no effect on the body weight of the vehicle-controls.

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. 1A shows OVA-specific IgG titers on oral TCDD-exposed 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. Similar results were observed in fecal OVA-specific IgA titers. On the other 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. 1B). 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. And, oral exposure of TCDD was a more sensitive immune response than nasally 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.

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

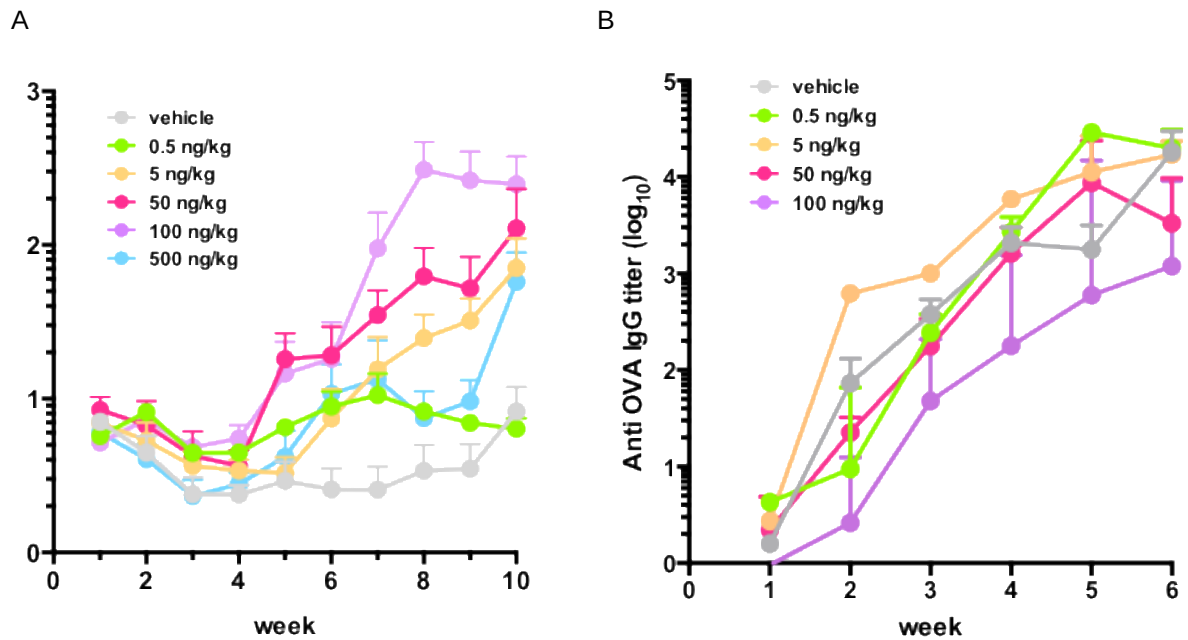


Fig. 1 Production of OVA-specific IgG in mice exposed with TCDD.

Mice were orally (A) or nasally (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).

**Acknowledgements**

This study was supported in part by a Grant-in-Aid (No. 16K09113) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**Reference**

1) Schecter A. *et al*; *Environ. Res*, 101, 419-28, 2006.
2) Chmill S *et al*; *Toxicol. Sci.*, 118, 98-107 2010.
3) Ishiwaka S *et al*; *J. Toxicol. Sci.*, 34, SP349-61 2009.
4) Yamada P *et al*; *Ecotoxicol. Environ. Saf.*, 84, 9-17 2012.
5) Zhao H *et al*; *Toxicology*, 325, 180-8 2014.