

# A Zinc Chelator TPEN Attenuates Airway Hyperresponsiveness and Airway Inflammation in Mice *In Vivo*

Satoru Fukuyama<sup>1</sup>, Yuko Matsunaga<sup>1</sup>, Wang Zhanghui<sup>1</sup>, Naotaka Noda<sup>1</sup>, Yukari Asai<sup>1</sup>, Atsushi Moriwaki<sup>1</sup>, Takafumi Matsumoto<sup>1</sup>, Takako Nakano<sup>1</sup>, Koichiro Matsumoto<sup>1</sup>, Yoichi Nakanishi<sup>1</sup> and Hiromasa Inoue<sup>1,2</sup>

## ABSTRACT

**Background:** Zinc is an essential element required for the cell metabolism, including gene transcription, signal transduction, immunity, and apoptosis. The pathophysiological role of zinc in asthma, however, is not entirely clear. Mast cells have been implicated in atopic asthma, and zinc deprivation has been reported to reduce mast cell activation. Here, we investigate the effects of a zinc chelator, N,N,N',N'-tetrakis (2-pyridyl-methyl) ethylenediamine (TPEN), on asthmatic responses in mouse models of ovalbumin (OVA)-induced airway hyperresponsiveness and allergic airway inflammation.

**Methods:** Mice were sensitized with OVA with or without the adjuvant aluminum hydroxide (alum) and subjected to OVA exposure with or without treatment of TPEN. Cell profiles and cytokine levels in bronchoalveolar lavage (BAL) fluids, airway responsiveness to inhaled acetylcholine, and goblet cell hyperplasia after allergen exposure were assessed.

**Results:** In mice sensitized to OVA without alum, TPEN significantly suppressed airway hyperresponsiveness and eosinophilia in BAL fluids. TPEN also attenuated the upregulation of TNF $\alpha$ , IL-13 and IL-4 in BAL fluids and goblet cell hyperplasia after OVA exposure. By contrast, in mice sensitized to OVA with alum, TPEN suppressed eosinophilia in BAL fluids but not airway hyperresponsiveness and goblet cell hyperplasia.

**Conclusions:** In pulmonary allergic inflammation induced in mice immunized with antigen without alum, zinc chelator inhibits airway inflammation and hyperresponsiveness. These findings suggest that zinc may be a therapeutic target of allergic asthma.

## KEY WORDS

airway hyperresponsiveness, asthma, zinc chelator TPEN

## INTRODUCTION

Bronchial asthma is a chronic inflammatory disease characterized by eosinophilic inflammation, airway hyperresponsiveness (AHR) to bronchospastic stimuli, and remodeling of the airways.<sup>1-3</sup> The airway inflammation in asthma is associated with a T helper (Th) 2-type immune response in which Th2 cell-derived cytokines, such as interleukin (IL)-4, IL-5, IL-9, and IL-13, are considered to contribute to eosinophil infiltration, AHR, and mucus hypersecretion.<sup>4,5</sup>

IL-4 and IL-13 also have critical roles in antigen-specific immunoglobulin (Ig) E production in atopic asthma, eliciting antigen-dependent and IgE-dependent mast cell and basophil activation.<sup>6</sup>

Mast cells are important effector cells in innate or acquired immunity and contribute to the development of allergic diseases, including atopic asthma. When mast cells are activated, potent inflammatory mediators are released in an antibody-dependent manner.<sup>7,8</sup> Fc $\epsilon$ RI $\alpha$ -deficient mice exhibit reduced airway inflammation and AHR in a mice allergic asthma

<sup>1</sup>Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka and <sup>2</sup>Present address: Department of Respiratory, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.  
Correspondence: Dr. Hiromasa Inoue, Department of Respiratory, Kagoshima University Graduate School of Medical and Dental Sci-

ences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan.  
Email: inoue-pulm@umin.net

Received 20 December 2009. Accepted for publication 27 September 2010.

©0000 Japanese Society of Allergy

model.<sup>9</sup>

Zinc is an essential element required for many cellular functions, especially immunity. Zinc-dependent biological function includes DNA replication, RNA transcription, signal transduction, enzymatic catalysis, redox regulation, cell proliferation, cell differentiation, and apoptosis.<sup>10-12</sup> Zinc is conventionally thought to protect airway epithelial cells as an antioxidant, an anti-inflammatory factor, and an inhibitor of apoptosis.<sup>13</sup> However, recent studies indicate that zinc deprivation inhibits CC chemokine production in human epithelial cells<sup>14</sup> and reduces mast cell activation<sup>15</sup> in bone marrow-derived mast cells. However, the pathophysiological role of zinc in allergic airway disease has not been clarified.

Although most mouse models of allergic airway inflammation involve sensitizing the mice with antigen admixed with aluminum hydroxide (alum), alum can enhance Th2 responses in the absence of IL-4 receptor  $\alpha$ , which is essential for IL-4 and IL-13 signals.<sup>16</sup> A recent study demonstrated that mast cells contribute significantly to the allergic airway inflammation induced by ovalbumin (OVA) challenge in mice sensitized with OVA without alum.<sup>17,18</sup> Mast cell granules are rich in zinc, which is essential for Fc $\epsilon$ RI-mediated activation and required for degranulation and cytokine production.<sup>15</sup> In the present study, we investigate the effects of a zinc chelator, N,N,N',N'-tetrakis (2-pyridyl-methyl) ethylenediamine (TPEN), on asthmatic responses *in vivo*.

## METHODS

### ANIMALS

Male C57BL6/J or BALB/c mice of 8 to 10 weeks of age were purchased from Japan SLC, Inc. (Shizuoka, Japan) and used in this protocol. All procedures and protocols were approved by the Kyushu University Animal Care and Use Committee.

### ZINC CHELATOR N,N,N',N'-TETRAKIS (2-PYRIDYL-METHYL) ETHYLENEDIAMINE (TPEN)

TPEN was prepared according to the method described previously.<sup>19</sup> Briefly, TPEN was dissolved in a vehicle consisting of ethanol: glycerol: H<sub>2</sub>O in a ratio of 1 : 3 : 6 and injected intraperitoneally 30 min before allergen exposure or IL-13 instillation.

### OVA SENSITIZATION AND CHALLENGE

C57BL6/J mice were sensitized with intraperitoneal injections of 10  $\mu$ g OVA (Grade V, SIGMA, St. Louis, MO, USA) without aluminum hydroxide (Thermo Scientific, Rockford, IL, USA) every other day for 14 days in a mast cell-dependent mice model of allergic asthma.<sup>20</sup> On days 40, 43, and 46, mice received an aerosol challenge containing either saline or 1% OVA for 30 min per day. Mice received an intraperitoneal injection of 10 mg/kg TPEN or vehicle solution 30 min before each OVA challenge. On day 47, 24 h after

the last aerosol challenge, measurements of the airway responsiveness and bronchoalveolar lavage (BAL) were performed. In a mast cell-independent model, mice were sensitized with intraperitoneal injections of 20  $\mu$ g OVA plus 2.25 mg alum on days 1 and 14 and challenged with 1% OVA solution for 30 min on days 28, 29, and 30. Mice received an intraperitoneal injection of 10 mg/kg TPEN or vehicle solution 30 min before each OVA challenge. On day 32, 24 h after the last aerosol challenge, measurements of the airway responsiveness and BAL were performed.

### INTRATRACHEAL INSTILLATION OF IL-13

BALB/c mice were injected with 2  $\mu$ g IL-13 intratracheally on days 1, 3, and 5 as described previously.<sup>21</sup> Mice received an intraperitoneal injection of TPEN or vehicle solution 30 min before each instillation. On day 6, measurements of the airway responsiveness and BAL were performed.

### MEASUREMENT OF AIRWAY RESPONSIVENESS

Mice were anesthetized, and their tracheas were cannulated via tracheostomy. The animals were ventilated to measure AHR to acetylcholine aerosol as described previously.<sup>21</sup> The airway opening pressure was measured with a differential pressure transducer and continuously recorded. Stepwise increases in the acetylcholine dose were given with an ultrasonic nebulizer. The data are expressed as the provocative concentration 200 (PC<sub>200</sub>), the concentration at which airway pressure was 200% of its baseline value, and PC<sub>200</sub> was calculated by log-linear interpolation for individual mice. Lower log PC<sub>200</sub> values represent greater AHR.

### BAL AND CELL COUNTING

Mice were given a lethal dose of pentobarbital, and lungs were gently lavaged one time with 1.0 ml saline via the tracheal cannula. Total cell counts and differential counts were performed.<sup>21</sup>

### HISTOLOGICAL ASSESSMENT

Lungs were fixed with 10% formalin, and tissue sections were stained with Periodic acid-Schiff/Alcian blue (PAS/AB) to assess goblet cell hyperplasia in the airways after antigen exposure.

### MEASUREMENT OF CYTOKINE LEVEL AND OVA-SPECIFIC IgE

Mouse IL-4 (Biosource, Camarillo, CA, USA), IL-13 (R&D Systems, Inc., Minneapolis, MN, USA), and TNF- $\alpha$  (Biosource) levels in the supernatant of BAL fluids were measured using ELISA kits. The concentration of serum OVA-specific IgE was measured using ELISA kits (Shibayagi Co., Ltd., Gunma, Japan).

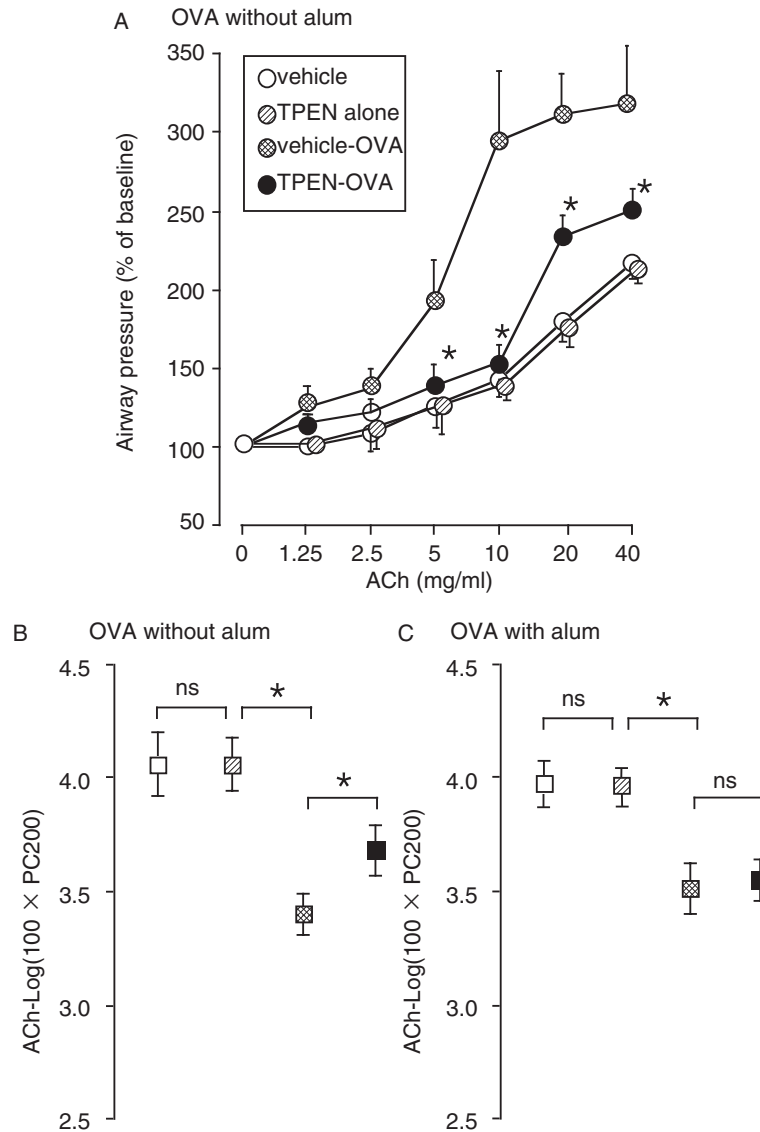


**Fig. 1** Effect of TPEN on airway responsiveness to inhaled acetylcholine (ACh) after OVA challenges. **A.** TPEN attenuates AHR to inhaled ACh in OVA sensitization without alum model. Airway responsiveness was determined by the ACh-dependent change in airway pressure. **B.** TPEN treatment before OVA challenges suppressed AHR after antigen challenges in sensitization without alum model. The provocative concentration 200 (PC<sub>200</sub>-ACh), at which airway pressure was 200% of its baseline value, is expressed as log (PC<sub>200</sub>-ACh × 100). Lower log PC<sub>200</sub> values represent greater AHR. **C.** Effect of TPEN on AHR to inhaled ACh in OVA sensitization with alum model. TPEN did not affect AHR after OVA exposure in this model. The values are the means ± SEM. *n* = 6 to 7. \**p* < 0.05. NS, not significant.

#### EFFECT OF TPEN ON EOTAXIN-1 LEVELS AFTER IL-13 STIMULATION IN VITRO

BEAS-2B cells, a human airway epithelial cell line, were cultured in DMEM/F-12 medium, with 10% FBS, penicillin, and streptomycin. The cells were treated with TPEN at indicated concentrations for 2

hours and were stimulated with human recombinant IL-13 (30 ng/ml). Twenty-four hours later, supernatant of cell culture was collected and eotaxin-1 levels were measured using ELISA kit (Biosource).

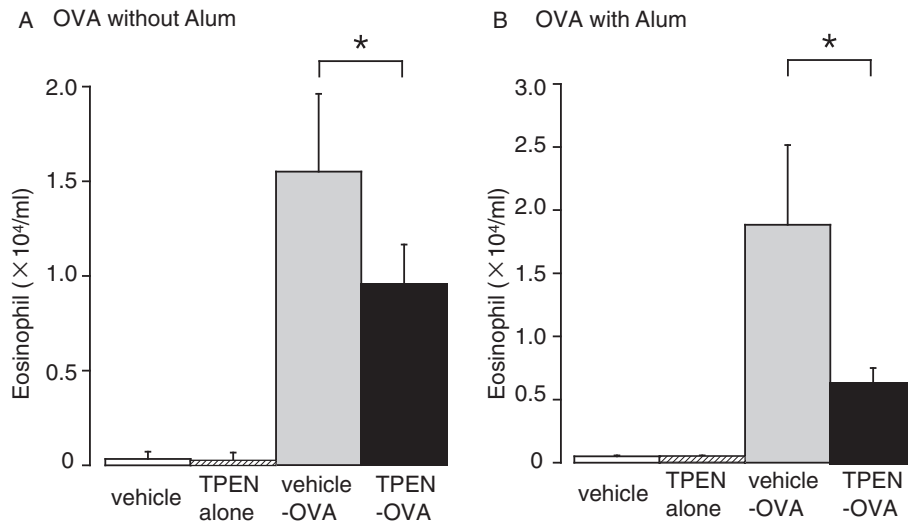


**Fig. 2** Effect of TPEN on eosinophil counts in BAL fluid after antigen challenges. **A.** Eosinophil counts in OVA sensitization without alum model. **B.** Eosinophil counts in OVA sensitization with alum model. TPEN treatment before antigen challenges suppressed eosinophilic inflammation in both model. Vehicle or TPEN solution was administered to OVA-sensitized mice 30 minutes before OVA challenges. BAL fluids were collected from vehicle alone, TPEN alone, vehicle-OVA, or TPEN-OVA mice. TPEN attenuates eosinophilia in BAL fluid.  $n = 5$  to  $7$ . \* $p < 0.05$ .

## DATA ANALYSIS

Values are expressed as the means  $\pm$  SEM. Differences between groups were analyzed by analysis of variance, and the significance of differences between values was assessed by Bonferroni correction. Values of  $p < 0.05$  were considered significant.

## RESULTS

### EFFECT OF TPEN ON ALLERGEN-INDUCED AIRWAY RESPONSES

In a mast cell-dependent model, OVA sensitization without alum and challenges resulted in AHR to inhaled acetylcholine. Treatment with TPEN during the effector phase of allergic responses attenuated AHR after OVA challenges (Figs. 1A, 1B). When alum was used for an adjuvant, AHR after OVA challenges was not affected by TPEN (Fig. 1C). These results suggest that TPEN affects AHR, at least in part, via a mast cell-dependent pathway. There were no significant differences in the baseline values of airway pressure among the control, TPEN alone, OVA challenge alone, and OVA plus TPEN.

### EFFECT OF TPEN ON EOSINOPHILIC INFLAMMATION

OVA sensitization and challenges resulted in an increased number of eosinophil in BAL fluids. TPEN treatment decreased eosinophil counts in BAL fluid after OVA challenges in allergic asthma models both with and without alum (Figs. 2A, 2B). TPEN did not change the number of macrophages, neutrophils, or

lymphocytes in BAL fluid after antigen challenges.

### EFFECT OF TPEN ON GOBLET CELL HYPERPLASIA

Histologically, eosinophil infiltration around the airway after allergen exposure was suppressed by TPEN treatment in both models sensitized with or without alum (data not shown). TPEN inhibited goblet cell hyperplasia after OVA challenges without alum model. On the other hand, TPEN treatment had no effect on goblet cell hyperplasia in sensitization with alum model (Fig. 3).

### EFFECT OF TPEN ON OVA-SPECIFIC IgE

The OVA-specific IgE concentration measured in sera after OVA challenges was significantly increased. Treatment with TPEN did not affect the serum OVA-specific IgE levels (Fig. 4). Thus, a zinc chelator administered only in the effector phase of allergic responses did not appear to influence the production of OVA-specific IgE.

### EFFECT OF TPEN ON CYTOKINE LEVELS IN BAL FLUID

We next measured the levels of Th2 cytokines, IL-4 and IL-13, and TNF $\alpha$ , because TNF $\alpha$  has been reported to contribute to an allergic airway inflammation in IgE-dependent and mast cell-dependent mouse models.<sup>18</sup> OVA challenge resulted in increases in these cytokines in BAL fluids. The levels of TNF $\alpha$ , IL-13 and IL-4 in BAL fluids were more re-

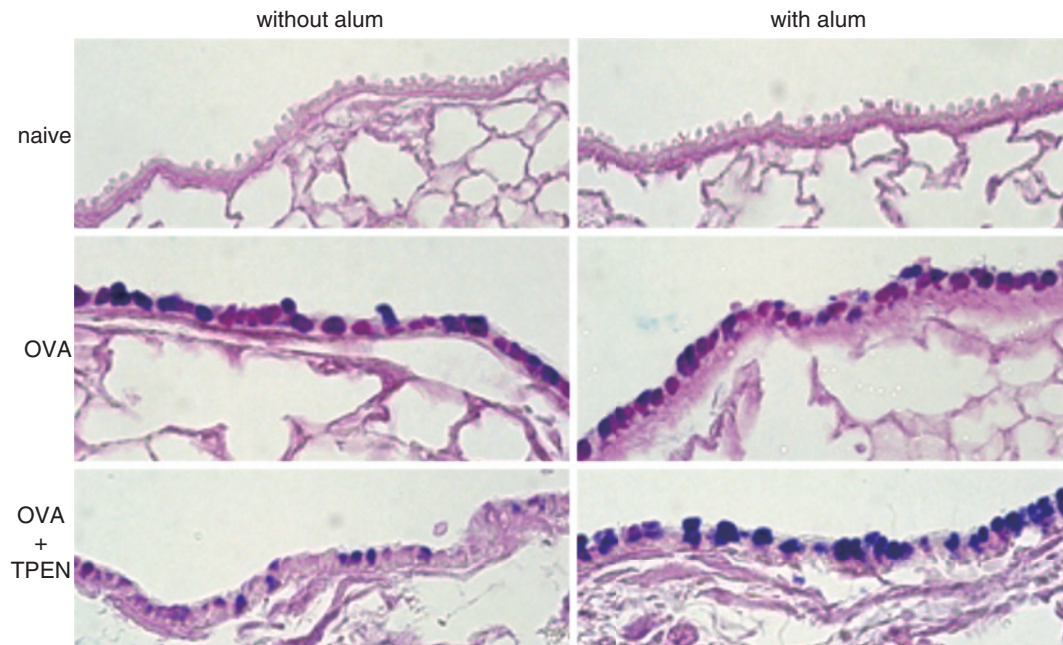


**Fig. 3** Effect of TPEN on goblet cell hyperplasia. Representative light photomicrographs of mouse lung tissue stained with Periodic acid-Schiff - Alcian blue (PAS/AB) to identify goblet cells (original magnification:  $\times 200$ ). There are marked increases in goblet cells in the epithelium of the intrapulmonary bronchus 24 h after OVA exposures. Treatment with TPEN caused marked decreases in the levels of PAS/AB staining cells after antigen challenges in mice sensitization without alum (Left panels). TPEN had no effect on the levels of PAS/AB staining cells after OVA challenges in mice sensitization with alum (Right panels).

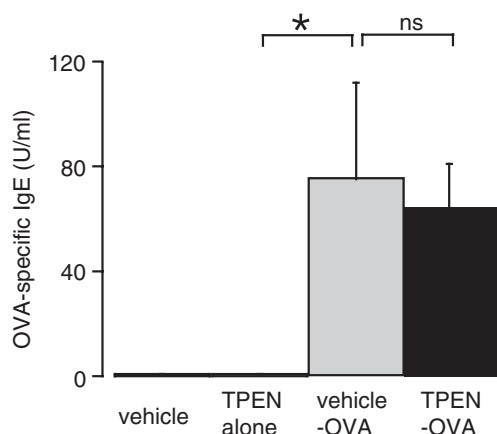


**Fig. 4** Effect of TPEN on serum OVA-specific IgE level after allergen exposure. TPEN did not affect OVA-specific IgE level after OVA challenges. Serum OVA-specific IgE was analyzed by ELISA.  $n = 5$  to  $7$ . NS, not significant.

duced in TPEN-treated mice than in vehicle-treated mice in allergic asthma model without alum (Fig. 5A). TPEN treatment reduced the upregulation of IL-13 levels in BAL fluids in asthma model sensitized with alum. On the other hand,  $\text{TNF}\alpha$  levels

were not affected by TPEN treatment in this model (Fig. 5B).

#### EFFECT OF TPEN ON EOSINOPHILIC INFLAMMATION AFTER IL-13 TREATMENT

The increase in eosinophils in BAL fluids after IL-13 instillation was suppressed by TPEN treatment (Fig. 6A). Furthermore, TPEN suppressed eotaxin-1 production from airway epithelial cells after IL-13 treatment *in vitro* dose-dependently (Fig. 6B). These results suggest that TPEN modulates chemokine and cytokine production from airway epithelial cells.

#### DISCUSSION

Although zinc is thought to protect airway cells, recent studies indicate that zinc contributes to mast cell activation<sup>15</sup> and chemokine production from epithelial cells.<sup>14</sup> The pathophysiological role of zinc in allergic airway responses has not been clarified. In the present study, we demonstrated that a zinc chelator, TPEN, attenuates AHR and eosinophilic inflammation in mast cell-dependent mice model of allergic asthma. These findings suggest that zinc is involved in allergic airway inflammation and hyperresponsiveness *in vivo*.

TPEN suppressed the upregulation of  $\text{TNF}\alpha$ , IL-13 and IL-4 in the airways. Th2-type cytokines, such as

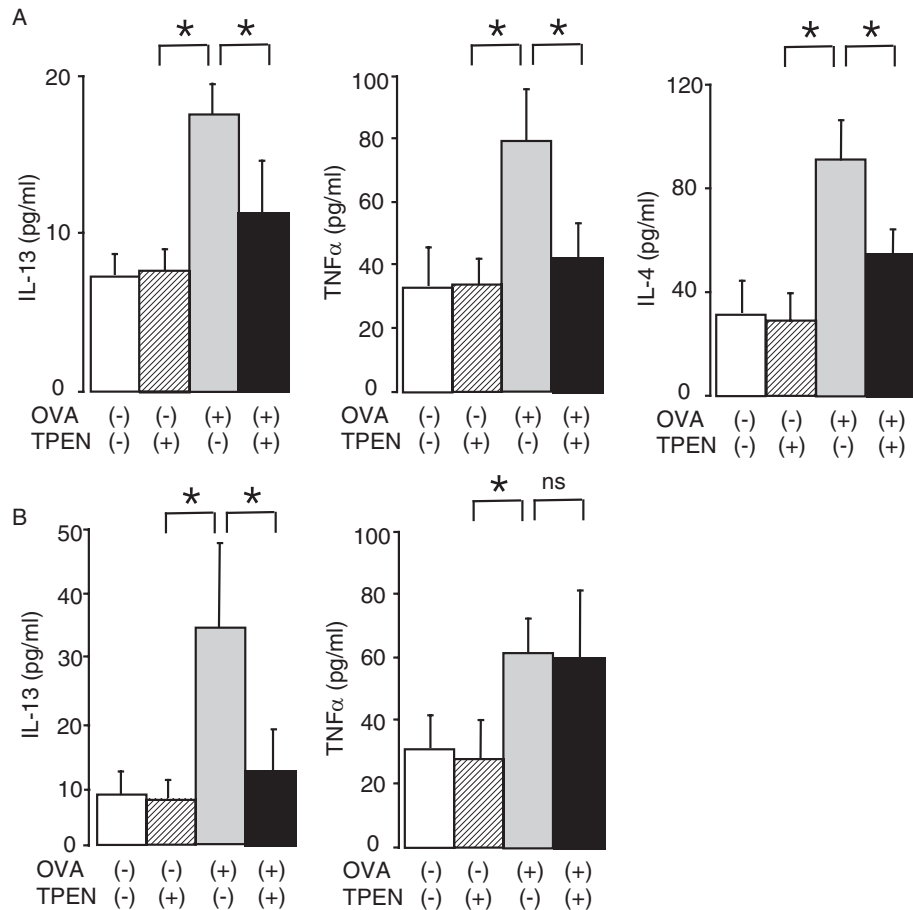


**Fig. 5** Effect of TPEN on cytokine levels in BAL fluids after antigen exposure. BAL fluids were obtained from vehicle alone, TPEN alone, or vehicle-OVA, TPEN-OVA mice. The cytokine profiles in BAL supernatants were analyzed by ELISA. **A.** Cytokine profiles in OVA sensitization without alum model. Elevated TNF- $\alpha$ , IL-13 and IL-4 levels in BAL fluids after allergen exposures were reduced in TPEN-treated mice.  $n = 7$  to  $9$ .  $*p < 0.05$ . **B.** Cytokine profiles in OVA sensitization with alum model. Elevated IL-13 but not TNF- $\alpha$  levels in BAL fluids after allergen exposures were reduced in TPEN-treated mice.  $n = 5$  to  $7$ .  $*p < 0.05$ . NS, not significant.

IL-4 and IL-13, have been implicated in the induction of AHR and allergic airway inflammation.<sup>4</sup> The importance of TNF $\alpha$ , especially from mast cells, in the manifestation of various phenotypes in mouse models of allergic asthma has been reported.<sup>17</sup> In addition to Th2 cells, mast cells can also produce IL-4. Because TPEN did not affect serum IgE concentration, TPEN might suppress the activation of mast cells during allergic airway responses.

Epithelial cells and fibroblasts are the main source of cytokines and chemokines in the airways of bronchial asthma. The zinc chelator inhibits the production of CC chemokine, such as eotaxin-1, eotaxin-2, RANTES, MCP-1, after TNF $\alpha$  treatment in lung epithelial cell and fibroblast *in vitro*.<sup>14</sup> After IL-13 treatment, the increase in eosinophils in BAL fluid was suppressed by TPEN treatment. Moreover, eotaxin-1

production from airway epithelial cells after IL-13 stimulation was attenuated by TPEN treatment *in vitro*. These results suggest that TPEN inhibits chemokine production after IL-13 instillation from airway epithelial cells.

Zinc signals are very important in many cell types of immune system, including dendritic cells, monocytes, mast cells, and T cells.<sup>22</sup> T cell function is susceptible to zinc deprivation and zinc signals activate protein kinase C in T cells<sup>23</sup> and promote IL-2-dependent T cell proliferation.<sup>24</sup> In allergic airway inflammation models, T cells have critical role in the development of AHR. In OVA sensitization with alum protocol, TPEN did not affect AHR, TNF $\alpha$  levels in BAL fluids and goblet cell hyperplasia after antigen challenges. On the other hand, TPEN attenuated AHR, TNF $\alpha$  levels and goblet cell hyperplasia after

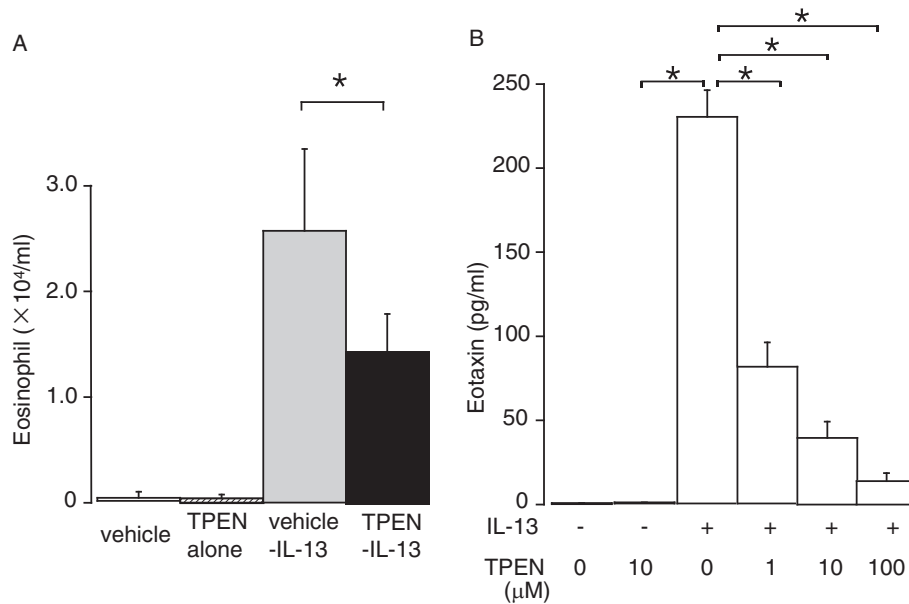


**Fig. 6** **A.** Effect of TPEN on eosinophil counts in BAL fluid after IL-13 instillation. Eosinophil counts were attenuated by TPEN treatment after IL-13 instillation *in vivo*.  $n = 5$  to  $7$ .  $*p < 0.05$ . **B.** Effect of TPEN on eotaxin-1 levels in the supernatant of airway epithelial cells after IL-13 treatment. TPEN dose-dependently suppressed eotaxin-1 production from BEAS-2B cells after IL-13 stimulation *in vitro*.  $n = 6$ .  $*p < 0.05$ .

OVA challenge in sensitization without alum protocol. The previous study demonstrated that mast cell and mast cell-derived  $\text{TNF}\alpha$  is essential for both AHR and eosinophilic airway inflammation in the model sensitized without alum, and not in the mice sensitized with alum.<sup>17</sup> The exact mechanism of the discrepancy between the inhibitory effects of TPEN on AHR and airway eosinophilia in mast cell-independent model is uncertain. Airway mucus hypersecretion contributes AHR.<sup>25</sup> Administration of  $\text{TNF}\alpha$  into the lungs of mice increases airway mucus gene expression,<sup>26</sup> and  $\text{TNF}\alpha$  induces the late-phase AHR.<sup>27</sup> The failure of TPEN to inhibit  $\text{TNF}\alpha$  might affect goblet cell hyperplasia and AHR under the condition of IL-13 suppression by TPEN.

A known adverse effect of TPEN is convulsions.<sup>28</sup> In general, the heavy metal chelator has neuronal cytotoxicity in the hippocampus.<sup>29</sup> Thus, a safer chelator or inhaled agent may be needed for clinical applications.

In conclusion, we provide evidence that TPEN attenuates AHR and airway inflammation induced by allergens in a mast cell-dependent mouse model. The extent to which our observations can be extended to human asthma remains to be determined. However, the present findings raise the possibility that a zinc chelator has potential therapeutic implications for bronchial asthma.

## ACKNOWLEDGEMENTS

We thank Makiko Umemoto, MSc, for technical as-

sistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and in part by the National Institute of Biomedical Innovation, Japan.

## REFERENCES

- Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature* 1999;**402** (Suppl):B12-7.
- Bousquet J, Chanez P, Lacoste JY *et al*. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;**323**:1033-9.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;**1**:520-4.
- Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;**17**: 255-81.
- Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;**22**:789-815.
- Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;**454**:445-54.
- Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 1999;**402** (Suppl):B24-30.
- Galli SJ. Complexity and redundancy in the pathogenesis of asthma: reassessing the roles of mast cells and T cells. *J Exp Med* 1997;**186**:343-7.
- Mayr SI, Zuberi RI, Zhang M *et al*. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. *J Immunol* 2002;**169**:2061-8.
- Truong-Tran AQ, Ruffin RE, Zalewski PD. Visualization of labile zinc and its role in apoptosis of primary airway epi-

- thelial cells and cell lines. *Am J Physiol Lung Cell Mol Physiol* 2000;**279**:L1172-83.
11. Prasad AS. Zinc: mechanisms of host defense. *J Nutr* 2007;**137**:1345-9.
  12. Overbeck S, Rink L, Haase H. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. *Arch Immunol Ther Exp (Warsz)* 2008;**56**:15-30.
  13. Zalewski PD, Truong-Tran AQ, Grosser D, Jayaram L, Murgia C, Ruffin RE. Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review. *Pharmacol Ther* 2005;**105**:127-49.
  14. Richter M, Cantin AM, Beaulieu C, Cloutier A, Larivee P. Zinc chelators inhibit eotaxin, RANTES, and MCP-1 production in stimulated human airway epithelium and fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L719-29.
  15. Kabu K, Yamasaki S, Kamimura D *et al*. Zinc is required for Fc epsilon RI-mediated mast cell activation. *J Immunol* 2006;**177**:1296-305.
  16. Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, Alexander J. Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* 1999;**163**:6448-54.
  17. Nakae S, Lunderius C, Ho LH, Schafer B, Tsai M, Galli SJ. TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. *J Allergy Clin Immunol* 2007;**119**:680-6.
  18. Nakae S, Ho LH, Yu M *et al*. Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice. *J Allergy Clin Immunol* 2007;**120**:48-55.
  19. Adler M, Dinterman RE, Wannemacher RW. Protection by the heavy metal chelator N,N,N',N'-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN) against the lethal action of botulinum neurotoxin A and B. *Toxicon* 1997;**35**:1089-100.
  20. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000;**192**:455-62.
  21. Kibe A, Inoue H, Fukuyama S *et al*. Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am J Respir Crit Care Med* 2003;**167**:50-6.
  22. Haase H, Ober-Blobaum JL, Engelhardt G *et al*. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. *J Immunol* 2008;**181**:6491-502.
  23. Haase H, Rink L. Functional significance of zinc-related signaling pathways in immune cells. *Annu Rev Nutr* 2009;**29**:133-52.
  24. Kaltenberg J, Plum LM, Ober-Blobaum JL, Honscheid A, Rink L, Haase H. Zinc signals promote IL-2-dependent proliferation of T cells. *Eur J Immunol* 2010;**40**:1496-503.
  25. Rogers DF. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr Opin Pharmacol* 2004;**4**:241-50.
  26. Busse PJ, Zhang TF, Srivastava K *et al*. Chronic exposure to TNF-alpha increases airway mucus gene expression in vivo. *J Allergy Clin Immunol* 2005;**116**:1256-63.
  27. Choi IW, Sun K, Kim YS *et al*. TNF-alpha induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A(2) activation. *J Allergy Clin Immunol* 2005;**116**:537-43.
  28. Lees GJ, Cuajungco MP, Leong W. Effect of metal chelating agents on the direct and seizure-related neuronal death induced by zinc and kainic acid. *Brain Res* 1998;**799**:108-17.
  29. Cuajungco MP, Lees GJ. Diverse effects of metal chelating agents on the neuronal cytotoxicity of zinc in the hippocampus. *Brain Res* 1998;**799**:97-107.