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RESEARCH ARTICLE

Pharyngeal aspiration of metal oxide nanoparticles showed potential of allergy aggravation effect to inhaled ovalbumin

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Abstract

The inhalation of manufactured metal oxide nanoparticles may lead to pulmonary toxicity. For instance, ZnO nanoparticles are known to induce pulmonary oxidative stress and inflammation. On the other hand, the pulmonary toxicity of TiO_2 nanoparticles is less than that of ZnO nanoparticles. Although, there have been some investigations concerning the induction of pulmonary oxidative stress and inflammation caused by manufactured metal oxide nanoparticles. And, although, it has reported that some nanoparticles cause aggravation of allergic reactions, there have so far been no reports regarding allergy aggravation effects of manufactured metal oxide nanoparticles. In this study, three types of nanoparticles, TiO_2, ZnO and SiO_2, were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice inhaled ovalbumin (OVA) a total of eight times over 3 weeks. After inhalation of OVA, the concentrations of total IgE, OVA-specific IgE and OVA-specific IgG_1 in serum increased in the mice treated with ZnO. TiO_2 and SiO_2 nanoparticles did not affect the OVA-specific IgE and IgG_1 levels. These results suggest that ZnO nanoparticles have the potential to aggravate allergic reactions. The results also suggest that Zn^{2+} release from ZnO nanoparticles is involved in the aggravation potential of allergies. However, pharyngeal aspiration of $ZnCl_2$ solution was not able to aggravate allergic reactions. Continuous Zn^{2+} release from ZnO nanoparticles to the lung is necessary for the aggravation of allergic reactions.

Keywords

Allergy aggravation, nanoparticle, ovalbumin, zinc oxide

History

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Introduction

A ''nanoparticle'' is defined as a ''nano-object with all three external dimensions in the nanoscale (size range from approximately 1 to 100 nm)'' (ISO/TS 27687:2008). Among nanoparticles, manufactured metal oxide nanoparticles are of great importance to industry; however, some types have exhibited toxicity. There are many studies regarding the pulmonary toxicity and cytotoxicity caused by metal oxide nanoparticles. For example, in rat models, the inhalation of NiO induced pulmonary inflammation (Mizuguchi et al., 2013) and the intratracheal instillation of NiO and ZnO nanoparticles induced pulmonary oxidative stress and inflammation (Fukui et al., 2012; Ho et al., 2011; Horie et al., 2012a). Additionally, some metal oxide nanoparticles have been shown to induce severe cytotoxic activity; namely, ZnO, CuO and NiO nanoparticles have been reported to cause large amounts of oxidative stress and cell death (De Angelis et al., 2013; Horie et al., 2012b; Karlsson et al., 2008; Syama et al., 2014). The toxic activity of a particular type of nanoparticle is largely dependent on its physical and chemical properties (Altunbek et al., 2014; Ho et al., 2011; Horie et al., 2012b; Ramasamy et al., 2014). One common chemical feature of nanoparticles is that they can release metal ions (Fukui et al., 2012; Horie et al., 2012b; Johnson et al., 2014; Kao et al., 2012). Nanoparticles, particularly metal oxide nanoparticles, have been shown to release metal ions when in biologically relevant fluids, such as culture medium. Specifically, it has been reported that ZnO, NiO and CuO nanoparticles release a larger amount of Zn^{2+}, Ni^{2+} and Cu^{2+} into culture medium than their fine-particle analogues (Horie et al., 2009a; Karlsson et al., 2009, 2014; Triboulet et al., 2014). Soluble nanoparticles are taken up into cells by endocytosis (Horie et al., 2009a) and, once internalized, they release metal ions. Intracellular metal ion release causes cell metabolism dysfunction and various cellular events, such as oxidative stress, apoptosis and cell death. Cellular uptake and intracellular metal ion release are common features of nanoparticle-specific cytotoxicity. Moreover, metal ion release is important not only *in vitro* but also *in vivo*. Metal ions released from metal oxide nanoparticles have been shown to cause pulmonary oxidative stress and inflammation (Fukui et al., 2012; Horie et al., 2011). The direct effects of manufactured nanoparticle inhalation, such as inflammation, lung injury and induction of oxidative stress, have been reported. On the other hand, it has been reported that diesel exhaust particles (DEP)

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Table 1. Properties of nanoparticles used in this study.

Materal	Code in this study	Surface treatment	Crystalline structure	Promary particle size (nm)	Specific surface area (m^2/g)
TiO_2	TiO_2	$Al(OH)_3$	Rutile	30–50	37.1
ZnO	ZnO			21	49.6
ZnO	ZnO–SiO_2	SiO_2		25	Unknown
SiO_2	SiO_2-A		Amorphous	7	300
SiO_2	SiO_2-B		Amorphous	34	80.0

These properties were obtained from manufacturer's data sheet.

include nanoparticles that enhance respiratory allergic reaction such as asthma (Takano et al., 1998). IL-4 expression was induced by exposure of DEP (Riedl et al., 2012; Saito et al., 2002). Additionally, some nanoparticles, such as silver and carbon black, showed the possibility of allergy aggravation effect (Chuang et al., 2013; Lefebvre et al., 2014). It has also been reported that co-exposure of Asian sand dust and Japanese cedar pollen enhanced nasal allergic reactions in guinea pigs (Ichinose et al., 2009). Four-time co-exposure to ovalbumin (OVA) and Asian sand dust aggravated the allergic inflammation of airways (He et al., 2013). Additionally, exposure to particulate matter with an aerodynamic diameter of less than 2.5 μm ($PM_{2.5}$) is associated with the aggravation of allergic reactions, such as asthma and conjunctivitis (Ichinose et al., 2008a; Mimura et al., 2014; Morishita et al., 2004; Nikasinovic et al., 2006). The presence of adsorbed materials on the surface of particulate matter is an important factor with respect to its potential to cause an adjuvant effect (Ichinose et al., 2008b). Some nanoparticles have the ability to adsorb large quantities of protein (Horie et al., 2009b) and form protein corona (Monopoli et al., 2011). Nanoparticles can adsorb environmental materials on their surface. These observations imply that nanoparticles have the potential to aggravate allergic reactions. However, the adjuvant effect on allergic reactions of manufactured metal oxide nanoparticles is still unknown. In this work, we have examined this potential.

Materials and methods

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of The University of Occupational and Environmental Health, Japan. The authorization reference number was AE11-008. Seven-week-old female C57BL/6 N mice were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The mice were fed a standard diet and they were allowed to acclimatize to their environment for 1 week before experimentation. Day/night schedule were every 12 h.

Nanoparticles and chemicals

The manufactured nanoparticles used in this study are listed in Table 1. In this study, four types of metal oxide nanoparticle were used: TiO_2, ZnO, SiO_2-coated ZnO and two types of SiO_2. TiO_2 (TTO-55(A)) and ZnO (FZO-50) nanoparticles were purchased from Ishihara Sangyo Kaisha Ltd. (Osaka, Japan). SiO_2-coated ZnO nanoparticles (Maxlight ZS-64) were purchased from Showa Denko K.K. (Tokyo, Japan). SiO_2 nanoparticles (UFP-80 and Reolosil QS-09) were purchased from Denki Kagaku Kogyo Kabushiki Kaisha (Tokyo, Japan) and Tokuyama Corporation (Tokyo, Japan), respectively. Crystalline silica (MIN-U-SIL5) was purchased from the US Silica Company (Berkeley Springs, WV). Aluminum hydroxide hydrate ($Al(OH)_3$ xH_2O) Gel Suspension (alum) was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan). The alum was dispersed in a 0.9% aqueous solution of NaCl at a concentration of 20 mg/mL. Chicken egg albumin was purchased from Seikagaku Corporation (Tokyo, Japan). Lipopolysaccharide (LPS) contamination of the OVA was assessed using an EndoLISA enzyme-linked immunosorbent assay (ELISA)-based endotoxin quantification assay kit (Hyglos GmbH, Bernried am Starnberger See, Germany). The OVA used in this work did not contain LPS. Zinc chloride ($ZnCl_2$) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nanoparticle powder was added to the PBS at a concentration of 1 mg/mL. The nanoparticles were then dispersed by sonication using a Bioruptor UCD-250 (Cosmo Bio Co. Ltd., Tokyo, Japan) for 2 min. The nanoparticle dispersions were used for aspiration immediately after preparation. Although nanoparticles formed aggregates in the PBS, there were no large aggregates that blocked the syringe at the aspiration.

Exposure of animals to nanoparticles

An outline of the exposure protocol is shown in Figure 1. The mice were divided into eight groups. Each group included five mice ($n = 5$). Fifty microliters of nanoparticle dispersion at a concentration of 1.0 mg/mL were administered to the lung by pharyngeal aspiration under anesthesia by intraperitoneal injection of pentobarbital sodium salt at a concentration of 8.1 mg/mL (Somnopentyl; Kyoritsuseiyaku Corporation, Tokyo, Japan. Eight times dilution by PBS). Anesthetized mouse was fixed on a cork board in the upright position. The dispersion was injected to pharynx by 0.5–200 μL gel-loading pipette tip attached to 1 mL tuberculin syringe (Terumo Corporation, Tokyo, Japan) under the lighting by the illuminated earpick (Asahi Electrochemical Co., Ltd., Osaka, Japan). Then aspiration of the dispersion by respiration was observed. Fifty microliters of PBS were also administered to lung by pharyngeal aspiration as a control. Additionally, 50 μL of $ZnCl_2$ at a concentration of 5.0 μg/mL was administered to lung by pharyngeal aspiration. In experiments 1 and 2, 1% OVA solution was administered by nasal exposure using a nebulizer (Porta-Neb: Medic-Aid Limited, Bognor Regis, UK). The picture of the exposure

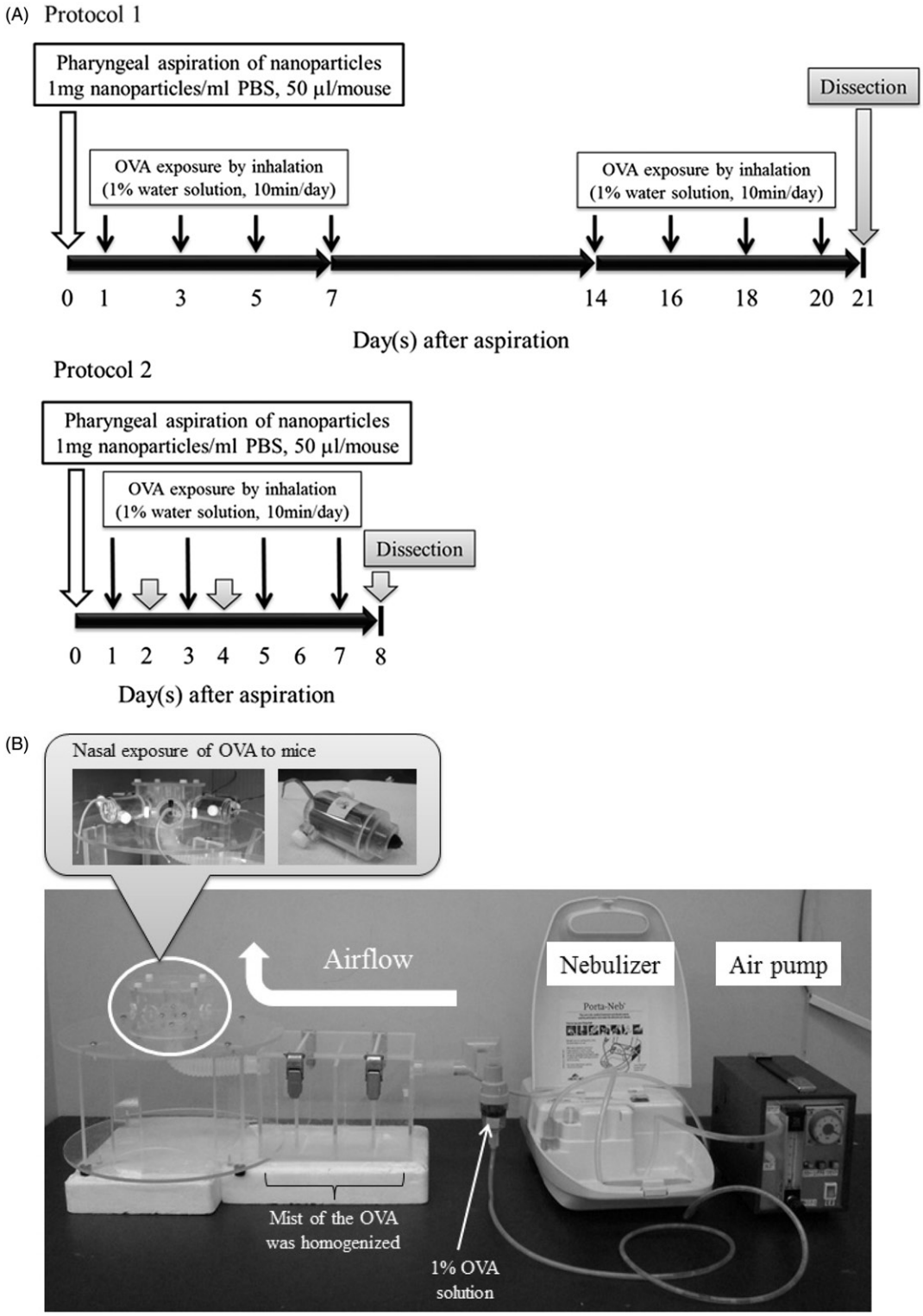

Figure 1. Experimental design of nanoparticle aspiration (A) and OVA inhalation. The photograph shows the OVA exposure system (B). Experiments 1 and 2 are conducted by protocol 1, and experiment 3 is conducted by protocol 2.

apparatus is shown in Figure 1. OVA solution was nebulized by the nebulizer and a mist of the OVA was homogenized. Then the mice were inhaled the mist by nasal exposure. Nasal exposure was carried out four times every other day for 10 min/day. After a break of 1 week, nasal exposure was carried out again four times every other day. In total, the mice were exposed to OVA eight times. The next day of the final exposure (21 days after aspiration), mice were anesthetized by intraperitoneal injection of pentobarbital sodium salt at concentration of 32.4 mg/mL. At autopsy, blood was taken from the heart and the lung was removed. In experiment 3, the pharyngeal aspiration was performed as in experiments 1 and 2. The mice were exposed to 1% of OVA solution four times every other day. During the OVA exposure period, mice were dissected and blood and lung were taken at 2, 4 and 8 days after aspiration.

Enzyme-linked immunosorbent assay

Serum was prepared by centrifuging whole blood at 5000 rpm for 5 min. Total IgE, total IgG, OVA-specific IgE and OVA-specific IgG_1 concentrations in serum were determined by ELISA. Total IgE concentration was determined using a Mouse IgE EIA Kit (Yamasa Choshi, Choshi, Japan). Total IgG was determined using a Mouse IgG total ELISA Ready-SET-Go! Kit (eBioscience, Inc., San Diego, CA). OVA-specific IgE was determined using a DS Mouse IgE ELISA (OVA) Kit (DS Pharma Biomedical Co., Ltd., Osaka, Japan). OVA-specific IgG_1 was determined with a Mouse Anti-OVA-IgG_1 ELISA Kit (Shibayagi Co., Ltd., Shibukawa, Japan). These ELISA experiments were performed according to the protocol of each manufacturer.

Real-time polymerase chain reaction

The lung was soaked in RNAlater RNA stabilization reagent (Qiagen, Hilden, Germany) immediately after removal. Lung tissue was homogenized with TissueRuptor (Qiagen) and then total RNA was prepared using an RNeasy Mini kit (Qiagen). cDNA synthesis was carried out with a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Thermo Fischer Scientific Inc., Waltham, MA). Real-time polymerase chain reaction (PCR) was conducted using a Step One real-time PCR system (Applied Biosystems), and PCR amplification of lung tissue was analyzed using TaqMan® gene expression assays (Life Technologies, Thermo Fischer Scientific Inc., Waltham, MA), with the mouse β-actin gene as an endogenous control. The code of the probes for TaqMan® gene expression assays for β-actin, heme oxygenase-1 (HO-1), metallothionein 1 (MT1), tumor necrosis factor (TNF-α), chemokine (C-X-C motif) ligand 2 (Cxcl2), interleukin 1 beta (IL-1β), cytochrome c oxidase (Cox2), nitric oxide synthase 2 (Nos2), arginase (Arg1) and prostaglandin E synthase (PTGES) were Mm00607939_s1, Mm00516005_m1, Mm00496660_g1, Mm00443260_g1, Mm00436450_m1, Mm00434228_m1, Mm03294838_g1, Mm00440502_m1, Mm00475988_m1 and Mm00452105_m1, respectively.

Statistical analysis

The data were expressed as the mean ± S.E. Statistical analyses were done via analysis of variance (ANOVA) using Dunnett tests for multiple comparisons. The calculation method is described in each figure legend.

Results

Influence of nanoparticle aspiration and subsequent OVA inhalation on IgE and IgG concentration in blood (Experiment 1)

After pharyngeal aspiration of nanoparticles, the mice inhaled OVA eight times in total (Figure 1). Pharyngeal aspiration of alum was also conducted as a positive control because the alum is known to have an adjuvant effect with respect to allergic reactions. Pharyngeal aspiration of PBS was also

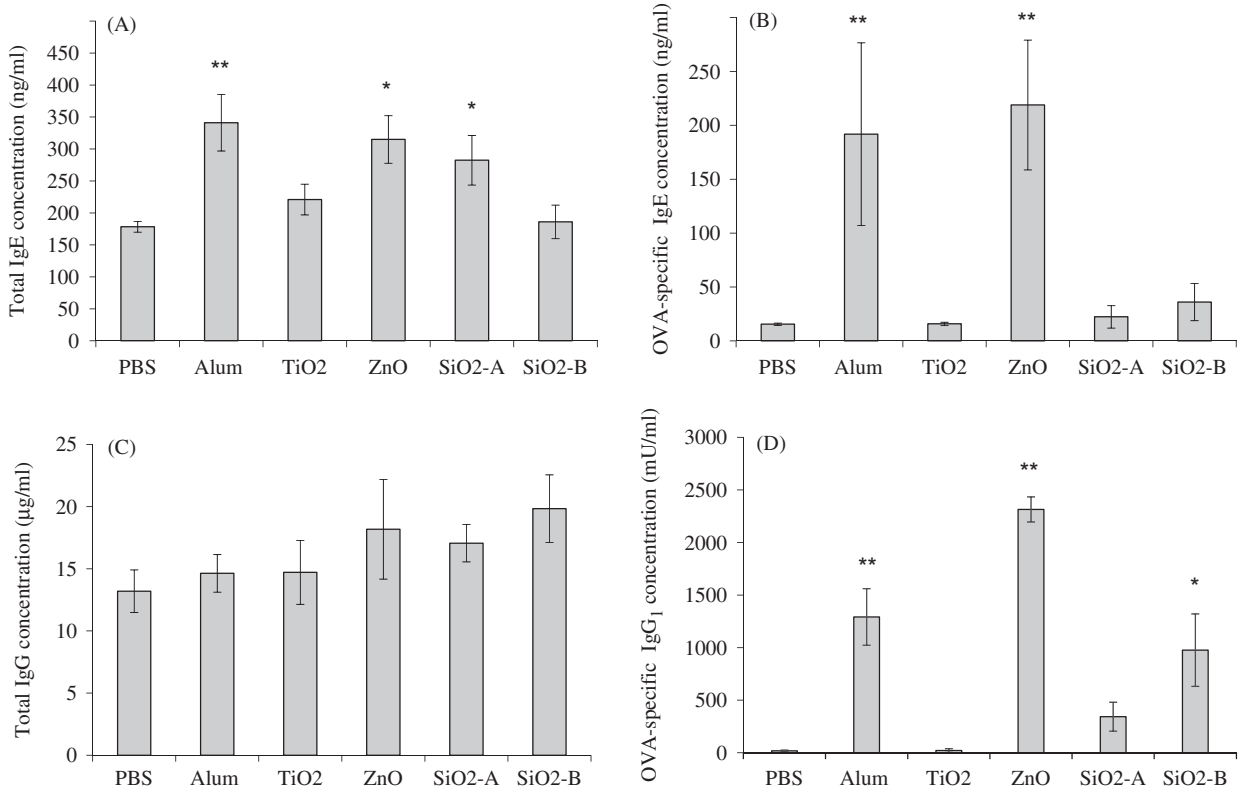

Figure 2. Concentration of total IgE (A), OVA-specific IgE (B), total IgG (C) and OVA-specific IgG_1 (D) in the blood of metal oxide nanoparticles-aspirated mice following OVA exposure (experiment 1). TiO_2, ZnO, SiO_2-A, SiO_2-B, alum and PBS were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice were exposed to OVA. The experimental design is shown in Figure 1. The values represent the mean ± SE. *$p < 0.05$, **$p < 0.01$ versus the PBS group, Dunnett, ANOVA.

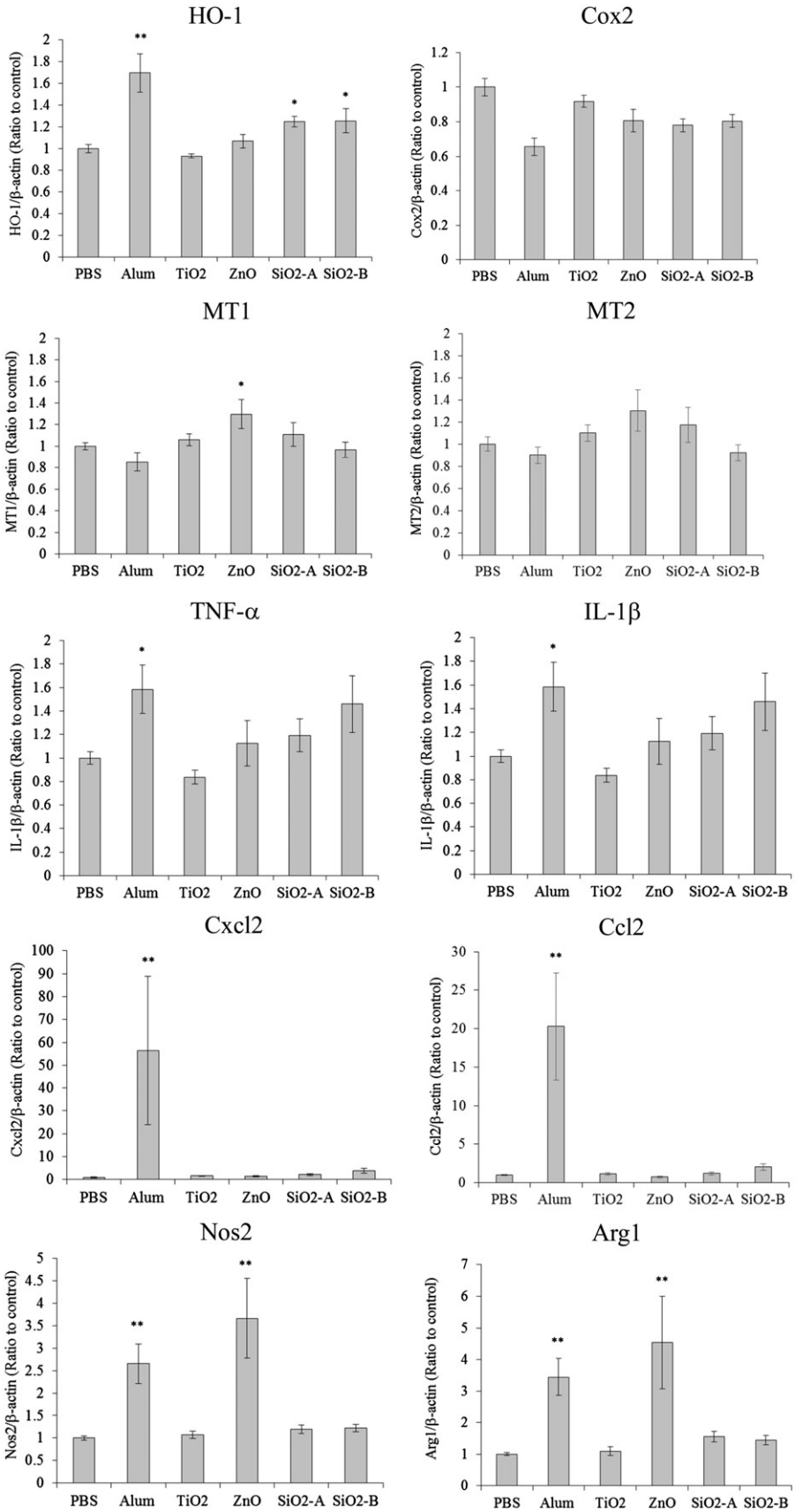

Figure 3. Gene expression in the lung tissue of metal oxide nanoparticle-aspirated mice following OVA exposure (experiment 1). TiO_2, ZnO, SiO_2-A, SiO_2-B, alum and PBS were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice were exposed to OVA. The experimental design is shown in Figure 1. Gene expression was determined by real-time PCR. The values represent the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$ versus the PBS group, Dunnett, ANOVA.

conducted as a negative control, and this group was not significantly different to the untreated animals that did not undergo pharyngeal aspiration and OVA exposure. The alum, ZnO and SiO_2-A groups showed an increase in IgE concentration in blood (Figure 2A). Additionally, the alum and ZnO groups showed a significantly increase in OVA-specific IgE (Figure 2B). In the ZnO group, the OVA-specific IgE level was approximately 12.3 times higher than in the

PBS group. In the alum group, the OVA-specific IgE level was approximately 12.7 times higher than that in the PBS group. An increase in OVA-specific IgE was not observed in the TiO_2 and SiO_2 groups. The total IgG concentration in blood did not increase due to pharyngeal aspiration of metal oxide nanoparticles (Figure 2C). The concentration of OVA-specific IgG_1 increased in the alum, ZnO and SiO_2-B groups. The OVA-specific IgG_1 level in the ZnO group was approximately 120 times higher than that in the PBS group. In the alum group, the OVA-specific IgG_1 level was approximately 70 times higher than that in the PBS group.

Influence of nanoparticle aspiration and subsequent OVA inhalation on gene expression in the lung (Experiment 1)

It has been reported that some manufactured metal oxide nanoparticles induced oxidative stress to culture cells and lung tissue (Fukui et al., 2012; Sarkar et al., 2014). For example, NiO nanoparticles caused induction of intracellular ROS level and induction of HO-1 expression (Horie et al., 2011). And intratracheal instillation of some nanoparticles caused pulmonary inflammation (Mizuguchi et al., 2013). Additionally, the suppression of inducible nitric oxide synthase (iNOS; NOS2) was involved in allergic effect of DEP (Saito et al., 2002). Therefore, we examined the association of oxidative stress, inflammation and allergic activity. After pharyngeal aspiration of nanoparticles and inhalation of OVA, lung tissue was obtained and the gene expression of stress response proteins, cytokines and differentiation markers was examined (Figure 3). Gene expression of the oxidative stress response protein HO-1 in the alum and SiO_2 groups was 1.2–1.7 times higher than it was in the PBS group. Expression of the metal response protein MT-1 gene in the ZnO group was increased 1.3 times compared to that in the PBS group. Gene expression of cytokines, TNF-a, Cxcl2 and IL-1b was increased in the alum group only. Expression of the Cox2 gene did not increase in any group. On the other hand, NOS2 and the Arg1 gene were increased significantly.

Influence of aspiration of ZnO nanoparticle and $ZnCl_2$ and subsequent OVA inhalation on IgE and IgG concentration in blood (Experiment 2)

Metal ion release from metal oxide nanoparticles is one of the main determining factors of nanotoxicity (Horie et al., 2012b,c). In particular, strong cytotoxicity has been shown to be caused by the cellular uptake of ZnO nanoparticles and subsequent intracellular Zn^{2+} release (Fukui et al., 2012). In this work, intratracheal administration of ZnO nanoparticles aggravated the allergic reaction to the inhaled OVA. Therefore, we next examined the relationship between Zn^{2+} and the aggravation effect. ZnO nanoparticles, SiO_2-coated ZnO nanoparticles, which showed lower solubility than ZnO nanoparticles and $ZnCl_2$ solution were administrated to mouse lungs by pharyngeal aspiration. Thereafter, the mice inhaled OVA eight times in total. Zn^{2+} concentrations in the ZnO and ZnO-SiO_2 dispersions were 5.3 and 2.4 μg/mL, respectively. On the basis of the Zn^{2+} concentration in the dispersed ZnO nanoparticles, an intratracheal administration of $ZnCl_2$ solution at a concentration of 5.0 μg/mL as Zn^{2+} was also conducted by pharyngeal aspiration. The OVA-specific IgE and OVA-specific IgG_1 concentrations in the blood of the ZnO and ZnO–SiO_2 groups were significantly higher than those in the PBS group (Figure 4). In the $ZnCl_2$ group, neither an increase in total OVA-specific IgE nor OVA-specific IgG_1 was observed.

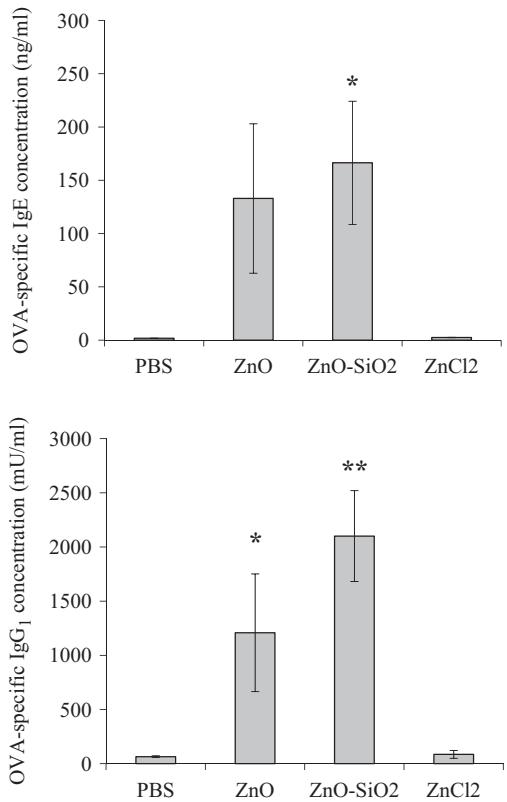

Figure 4. Concentration of OVA-specific IgE and OVA-specific IgG_1 in the blood of zinc compound-aspirated mice following OVA exposure (experiment 2). ZnO, ZnO–SiO_2, $ZnCl_2$ and PBS were administered to mouse lung by pharyngeal aspiration. The mice were then exposed to OVA. The experimental design is shown in Figure 1. The values represent the mean ± SE. $*p<0.05$, $**p<0.01$ versus the PBS group, Dunnett, ANOVA.

Influence of aspiration of ZnO nanoparticle and $ZnCl_2$ and subsequent OVA inhalation on gene expression in the lung (Experiment 2)

After pharyngeal aspiration of ZnO, ZnO–SiO_2 nanoparticles and $ZnCl_2$ solution and subsequent OVA inhalation, lung tissue was obtained and gene expression of stress response proteins, cytokines and differentiation markers was examined (Figure 5). In the ZnO aspiration group, the expression of Arg1, MT1 and MT2 genes increased significantly. In the ZnO–SiO_2 group, the expression of MT1, MT2 and Cox2 genes increased significantly. The pharyngeal aspiration of $ZnCl_2$ solution did not affect the expression of any genes examined in this study.

Influence of ZnO nanoparticles on allergic response early in the OVA inhalation period (Experiment 3)

The influence of ZnO nanoparticles on the lung early in the OVA inhalation period was also examined (experimental design is shown in Figure 1). The OVA-specific IgE and

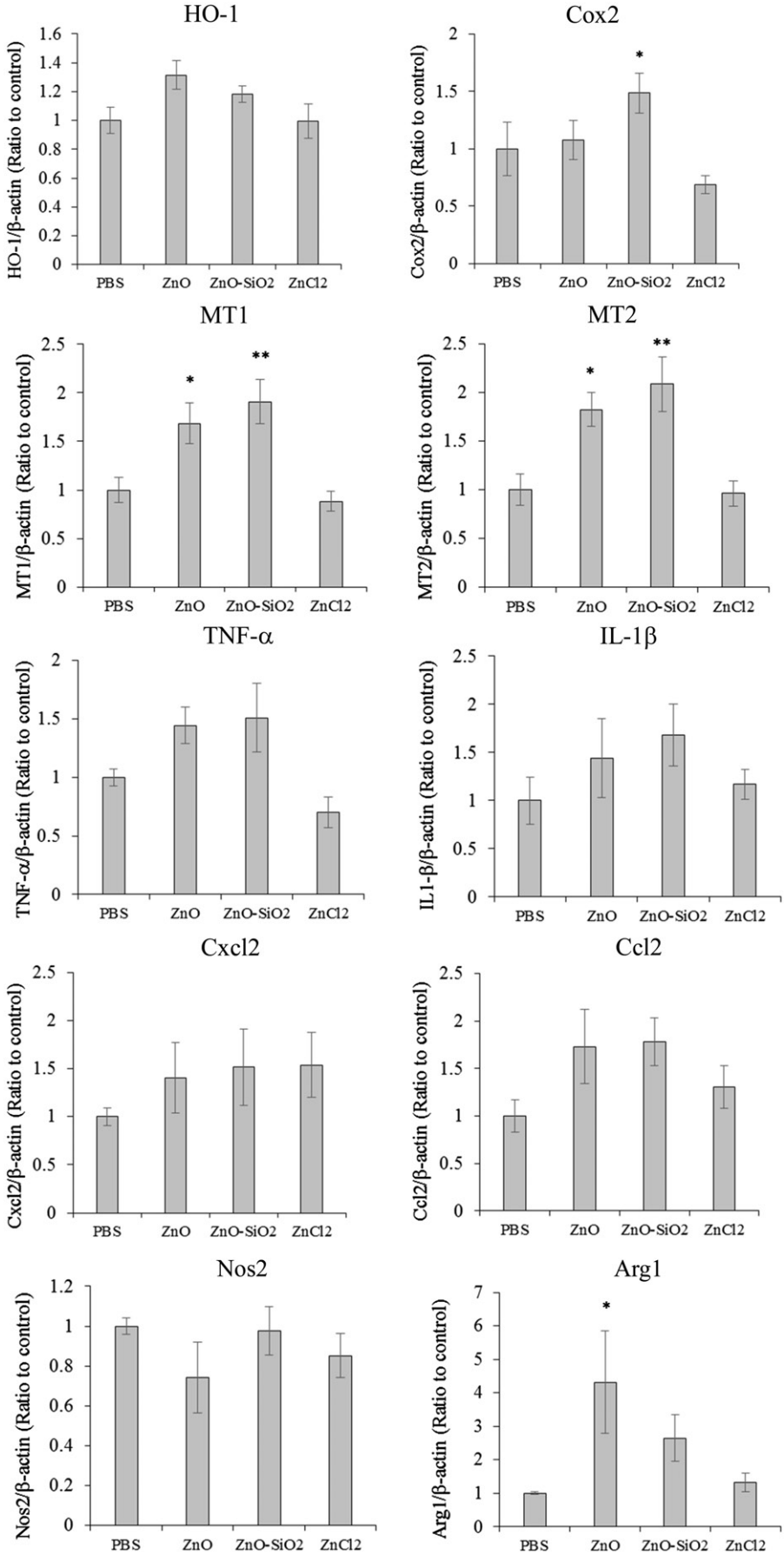

Figure 5. Gene expression in the lung tissue of zinc compound-aspirated mice following OVA exposure (experiment 2). ZnO, ZnO–SiO_2, $ZnCl_2$ and PBS were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice were exposed to OVA. The experimental design is shown in Figure 1. Gene expression was determined by real-time PCR. The values represent the mean ± SE. $*p<0.05$, $**p<0.01$ versus the PBS group, Dunnett, ANOVA.

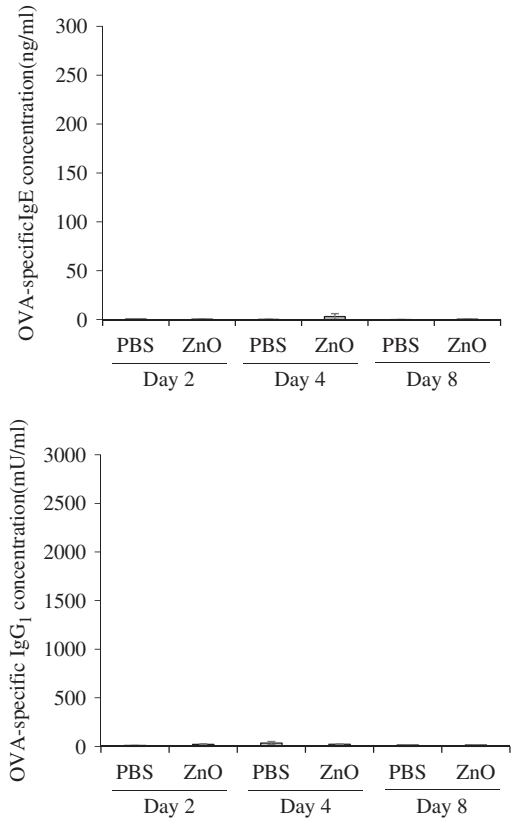

Figure 6. Concentration of OVA-specific IgE and OVA-specific IgG_1 in the blood of zinc compound-aspirated mice following OVA exposure (experiment 3). ZnO and PBS were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice were exposed to OVA. The experimental design is shown in Figure 1. The values represent the mean ± SE. No significant differences were observed.

OVA-specific IgG1 in blood after one, two or three inhalations of OVA were measured. The OVA-specific IgE and OVA-specific IgG1 in blood did not increase at any time points (Figure 6). Gene expression of HO-1 in the lung in the ZnO group was higher than that in the control group 2 days after aspiration, although not significantly (Figure 7). Four days after aspiration, gene expression of HO-1 in the lung in the ZnO group was significantly higher than that in the control group. Expression of MT1 and MT2 genes increased in the ZnO group. Additionally, the expression of TNF-α and Cxcl2 genes increased in the ZnO group. A much greater expression of Arg1 gene was observed in the ZnO group 2 and 4 days after aspiration.

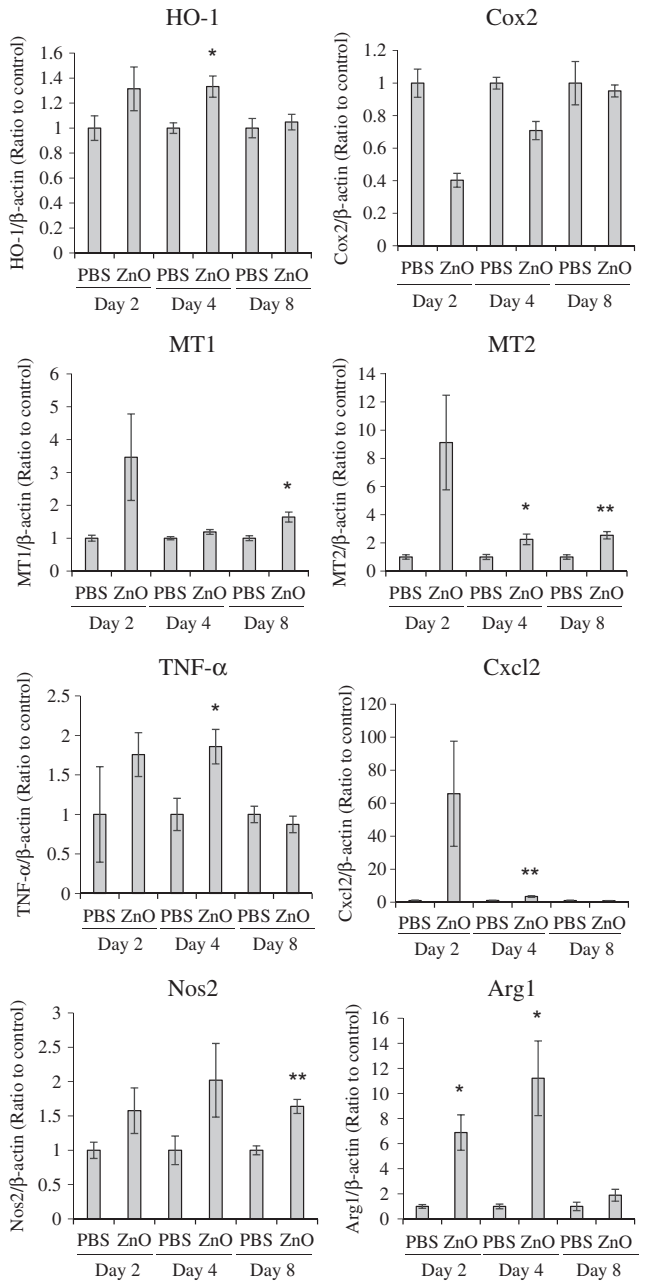

Figure 7. Gene expression in the lung tissue of zinc compound-aspirated mice following OVA exposure (Experiment 3). ZnO and PBS were administered to mouse lungs by pharyngeal aspiration. Subsequently, the mice were exposed to OVA. The experimental design is shown in Figure 1. Gene expression was determined by real-time PCR. The values represent the mean ± SE. $*p<0.05$, $**p<0.01$ versus the PBS group, Dunnett, ANOVA.

Discussion

In this study, nanoparticles were administered to the lung by pharyngeal aspiration and subsequently OVA was administered. After eight inhalations of OVA, OVA-specific IgE and OVA-specific IgG_1 levels in blood were increased in the ZnO group relative to the PBS group. These results suggest that inhalation of ZnO nanoparticles has the potential to aggravate allergic reactions. However, not all types of nanoparticle resulted in an allergy aggravation potential. The aggravation effect was not observed in the TiO_2 and SiO_2 groups, only in the ZnO and ZnO–SiO_2 groups. The observed increase in anti-OVA IgE was similar to the alum group, which was used as a positive control. However, although the administered dose of alum was 1000 μg/animal, the dose of ZnO nanoparticles was 50 μg/animal. At least, ZnO nanoparticles showed an allergy aggravation potential similar to alum. However, we did not examine the dose response of the allergy aggravation potential caused by the ZnO nanoparticles in this study. Thus, there is no meaning to compare them. It is well-known that the down regulation by strong stimulation is easy to occur in the allergic response.

Metal ions released from ZnO nanoparticles play an important role in events related to cytotoxicity and pulmonary

toxicity, such as cell membrane damage, induction of inflammation and induction of oxidative stress (Fukui et al., 2012; Horie et al., 2012b; Johnson et al., 2014; Kao et al., 2012; Ramasamy et al., 2014). Since administration of ZnO nanoparticles to the lung showed an allergy aggravation potential in this work, the role of Zn^{2+} in causing the effect was examined. Gene expression of metallothionein in lungs 21 days after the administration of ZnO nanoparticles was measured. The results suggest that Zn^{2+} was continuously released from the ZnO into the lung. The allergy aggravation potential was seen in the ZnO and ZnO–SiO_2 groups. However, it was not observed in the $ZnCl_2$ group. These results suggest that it is not only the presence of Zn^{2+} but also the ZnO nanoparticle as source of Zn^{2+} that is an important factor in the allergy aggravation potential. Although the expression of metallothionein was increased in the ZnO and ZnO–SiO_2 groups 21 days after administration, it did not increase in the $ZnCl_2$ group. We previously reported that when ZnO nanoparticles dispersion and $ZnCl_2$ solution, which were included same concentration of Zn^{2+}, were injected to rat lung by intratracheal instillation, gene expression of MT1 in lungs was increased. The gene expression of MT1 was remarkably induced at 24 h after instillation of ZnO nanoparticles and $ZnCl_2$. However, at 72 h after instillation, induction of MT1 gene expression was observed in only ZnO nanoparticles injected animals (Fukui et al., 2012). On the basis of the measurements made 21 days after administration, we concluded that oxidative stress did not occur in the lung because gene expression of HO-1 had not increased. And inflammation cytokines and chemokines had also not increased. On the other hand, gene expression of HO-1, TNF-α and Cxcl2 in lung had increased in the ZnO group when measured 4 days after administration. At this time, OVA-specific IgE and IgG_1 had not yet increased. Although Zn^{2+} may cause oxidative stress, on the other hand, Zn^{2+} also enhances expression of metallothionein. The metallothionein has anti-oxidative stress activity. It has been reported that the protective effect of MT was about five times better than that of glutathione (GSH) (Cai et al., 1998). Thus, there is a possibility that the oxidative stress was reduced by the expression of metallothionein. Therefore, there is the possibility that the allergy aggravation potential of the ZnO nanoparticles was caused by early oxidative stress and subsequent inflammation. Continuous Zn^{2+} release may be associated with the allergy aggravation potential of the ZnO nanoparticles. In order to understand the mechanism of the allergy aggravation potential completely, further examination, such as the analysis of neutrophils, macrophages and cytokines in bronchoalveolar lavage fluid (BALF) will be necessary.

Cellular uptake by endocytosis and subsequent intracellular release of Zn^{2+} are important factors with respect to the cytotoxicity of ZnO nanoparticles (Fukui et al., 2012; Gilbert et al., 2012; Ramasamy et al., 2014). ZnO nanoparticles serve as the Zn source. Also important are the retention time and Zn source. The allergic aggravation effect of ZnO may be dependent on the amount of Zn^{2+} released and the retention time of the ZnO nanoparticles. The TiO_2 nanoparticles used in this study are insoluble (Horie et al., 2012b). The allergy aggravation potential caused by insoluble nanoparticles such as TiO_2 may be negligible.

On the other hand, it has been reported that the bacteria and chemicals adsorbed onto the particles aggravate pollen allergies (Ichinose et al., 2009). Many types of nanoparticles have the ability to adsorb large amounts of material. The TiO_2 nanoparticles used in this study have a large protein adsorption ability (Horie et al., 2009b). Although, TiO_2 and ZnO nanoparticles used in this study had similar protein adsorption ability (Horie et al., 2009b), only ZnO nanoparticles showed allergy aggravation activity. Although, we did not examine materials adsorbed on the TiO_2 nanoparticles in this study, the ''adsorption ability,'' which may be dependent on the specific surface area of a nanoparticle, did not affect the allergy aggravation potential. Eventually, research into the influence of adsorbed materials on the allergy aggravation potential of nanoparticles will be necessary.

On the other hand, it has been reported that the inhaled nanoparticles were deposited not only in the respiratory tract but also in the nasal passages (Garcia & Kimbell, 2009). In the present study, we did not examine an influence of the manufactured metal oxide nanoparticles deposited in the nasal passages for the allergy aggravation potential. It may be necessary to consider the influence of deposition to the effect of allergic aggravation on the nasal cavity of nanoparticles.

In conclusion, it was shown that nanoparticles have the ability to aggravate allergic reactions. Although, nanoparticles are not antigens, there is the possibility that allergic reactions, such as asthma, atopic dermatitis and pollen allergies, are aggravated by nanoparticle inhalation. On the other hand, the mechanism of the allergy aggravation potential of manufactured nanoparticles is not understood completely. It is necessary to clarify the mechanism to evaluate the allergy aggravation potential. As the next step, analysis of eosinophils in the lung and inflammatory mediators related to Th2 are necessary.

Declaration of interest

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