



ortho-Phthalaldehyde enhances allergen-specific IgE production without allergen-specific IgG in ovalbumin-sensitized mice

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ABSTRACT

ortho-Phthalaldehyde (OPA) is commonly used as a safer and more effective chemical disinfectant for use with medical devices in hospitals. However, the cases of patients with occupational bronchial asthma or contact dermatitis are recently reported among workers in the medical professions who were exposed to OPA disinfectant. Mechanism of allergic reaction associated with OPA is poorly understood. The purpose of this study is that OPA may act as an immunological adjuvant in the allergic reaction accompanied by enhanced specific-IgE production in response to allergen challenge in OVA-sensitized mice. OPA induced increase of total cell numbers, and reflected infiltration of neutrophils in BAL fluid after allergen challenge in sensitized mice, dose-dependently. However, total protein concentration in BAL fluid did not change in the all of groups. The OPA induced up-regulation of eotaxin and monocyte chemoattractant protein-1 mRNAs in the lung as well as the increase in OVA-specific IgE in sensitized mice compared with non-sensitized controlled mice without increase in the level of OVA-specific IgG. Cytokines IL-4 and IL-5 mRNA were expressed by allergen (OVA) challenge in both lungs collected from OPA-administrated-sensitized and OPA-administrated-nonsensitized mice. From these data, we concluded that low concentration of OPA that enhanced the OVA-induced recruitment of neutrophils to the lung and the production of allergen-specific IgE, suggesting that OPA acts as an immunological adjuvant.

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1. Introduction

Glutaraldehyde (GTA) has been used as a disinfectant for medical devices for over 30 years, however serious health problems associated with GTA among medical workers were reported (Ballantyne and Jordan, 2001; Takigawa and Endo, 2006). Skin irritation and sensitization, and allergic contact dermatitis due to sterilization of medical equipments using GTA are well documented. GTA is readily volatile at the ambient temperature, and its pungent vapor irritates eye and respiratory tract. Therefore, the cases of occupational asthma induced by GTA vapor have been reported in the area where endoscopy nurses and respiratory technologists work.

ortho-Phthalaldehyde (OPA) has been used as a potential alternative compound to GTA in the past 10 years, as a high-level disinfectant agent for endoscope cleaning. OPA has some advantages to GTA, e.g. low volatility, fast and superior antimicrobicidal activity, and no need for activation. Therefore, OPA has been increas-

ingly used as a safer and more practical alternative to GTA. Recently, Suzukawa et al. (2006, 2007) reported that a patient who had cedar pollen rhinitis experienced anaphylaxis following laryngoscopy disinfected by OPA. *In vitro* histamine-release tests using this patient's basophils revealed that OPA evoked obvious release of histamine. In addition to this case, Sokol (2004) reported that severe anaphylactic reactions were observed in 4 patients following cystoscopy sterilized with OPA instead of GTA. Total plasma IgE level was slightly elevated in one of these patients. These evidences strongly indicated that OPA might enhance allergic reaction in the subject who was sensitized to a certain allergen. Although OPA has lower volatility than GTA, a case of occupational bronchial asthma caused by OPA vapor during sterilization of endoscopies in medical workplace has been also reported (Fujita et al., 2006). In addition, there are 189 reports about health effects caused by OPA in Manufacturer and User Facility Device Experience (MAUDE) Database compiled by US FDA through September 2008. Five reports mentioned allergic reactions (i.e. asthma, difficulty in breathing, lymph node enlargement, etc.) in 10 reports in 2008.

These cases strongly suggested that aldehyde, OPA similar compound to GTA appeared to act as immunological adjuvant and elicited allergic reactions in sensitized subjects. For this reason, this study was conducted to describe the mechanism of OPA-associated immunological reaction in ovalbumin (OVA)-sensitized mice.

Abbreviations: OPA, ortho-phthalaldehyde; GTA, glutaraldehyde; OVA, ovalbumin; BAL, bronchoalveolar lavage; RT-PCR, reverse transcriptase-polymerase chain reaction; PBST, PBS with 0.05% Tween-20; MCP-1, monocyte chemoattractant protein-1; TNF- α , tumor necrosis factor- α ; IL, interleukin.

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2. Materials and methods

2.1. Experimental animals

Female ICR mice aged 8–10 weeks were obtained from CLEA Japan Inc. (Tokyo, Japan). Animals were housed in individual cages supplied with HEPA-filtered air. The ambient temperature in a chamber was controlled at $23 \pm 1^\circ\text{C}$ and the relative humidity was maintained from 50 to 60%. The light cycle in the room was 7 a.m.–7 p.m. Food and water was provided *ad libitum* throughout the experiments. All animals were conducted in accordance with the guidelines provided by Kurume University Institutional Animal Care and Use Committee under an approved protocol.

2.2. Experimental protocol (Fig. 1)

Animals were divided six groups; OPA + OVA group, OPA group, OVA group, PBS group, Alum + OVA group and Alum group. The OPA + OVA, Alum + OVA and OVA groups were injected s.c. with 10 μg of ovalbumin (OVA; grade V, Sigma–Aldrich Co., St. Louis, MO, USA) suspended in 200 μl sterile PBS, on days 0 and 5. The OPA + OVA and OPA groups were injected s.c. with 200 μl of OPA (WAKO, Tokyo, Japan; 0.0025, 0.0125, 0.025, 0.125, 0.25%) on days 0 and 5. For OPA–OVA group, OPA and OVA were mixed prior to injection, and mixture was injected immediately. Precipitate due to polymerization of OVA was not observed in the mixture. The Alum + OVA group was injected s.c. 10 μg of OVA emulsified with 4 mg of sterile aluminum hydroxide (Alum, Alu-Gel-S Suspension, Serva Electrophoresis GmbH, Heidelberg, Germany) on days 0 and 5. The PBS group was administered 200 μl of sterile PBS. Thirteen or fourteen days after the last injection, all of mice was challenged with 5% OVA saline solution using an ultrasonic inhaler (Spiral 3621 Mark V, Medisonic USA, Clarence, NY, USA) for 20 min in a whole body exposure chamber (Ichiki et al., 2005). Six to twelve mice were used in all groups.

2.3. Sampling

Three hours after the last antigen challenge, all animals were anesthetized by pentobarbital (25 mg/kg body weight, i.p.), and blood was collected from the right ventricle. A catheter was inserted into the trachea and immobilized; then bronchoalveolar lavage (BAL) fluid was collected immediately, and bronchoalveolar spaces were rinsed 3 times with 0.5 ml sterile saline (5 ml/100 g body weight) via the inserted catheter. The BAL cells were counted cell number with Trypan blue staining. Aliquots of cells were fixed onto glass slide using Cytospin II (Thermo Shandon Ltd., Cheshire, UK), and then stained by Diff-Quick (Sysmex International Reagents Co., Ltd., Kobe, Japan), for differential cytology under a light microscopy by counting minimum of 200 cells. BAL fluids were centrifuged at $1500 \times g$ for 5 min; supernatants were stored -40°C until analysis. Total protein in BAL fluid was measured by Lowry et al. (1951) method. A part of right lung tissue preserved in RNAlater (Ambion Inc., TX, USA), and stored under -20°C until analysis.

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Lung tissues were homogenized in RNA later using a Polytron PT3100 (Kinematica, Littau, Switzerland). The total RNA was isolated from homogenate by using RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany). Both quantity and quality of RNA was estimated by a bioanalyzer (Agilent Technologies 2100 Bioanalyzer, Waldbronn, Germany). The first strand cDNA was synthesized using SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Up to the synthesis of cDNAs, all experiments were carried out under RNase-free condition. First-strand cDNAs were then used as templates in PCR. The oligonucleotide primers synthesized are summarized in Table 1 (Suganuma et al., 2002; Zager, 2005; Krzesicki et al., 1997; Fung et al., 1997; Lee et al., 2008). PCR was carried out with the following experimental conditions: denaturation at 95°C for 30 s, annealing at optimized temperature for 1 min, and extension at 72°C for 1 min for each cycles as mentioned in Table 1. PCR products were electrophoresed and visualized with Agilent 2100 Bioanalyzer.

2.5. Measurement of antigen-specific IgE and IgG in sera

Ovalbumin-specific IgE and IgG concentrations were measured by ELISA. OVA (1 $\mu\text{g}/\text{ml}$) was coated onto 96-well plate overnight at 4°C in 50 mM pH 9.6 carbonate-bicarbonate buffer. The plates were blocked with PBS containing 1% BSA for 1 h. After the platelet was washed with PBS with 0.05% Tween-20 (PBST), various serum samples were added to the wells, incubated at room temperature for 2 h. After washing the wells, biotinylated anti-mouse IgE monoclonal antibody (clone: HMK-12, Yamasa Corp., Chiba, Japan) was added to each well. After incubation for 1 h, streptavidin- β -D-galactopyranosidase was added and incubated for 1 h. 4-methylumbelliferyl- β -D-galactopyranoside (0.1 mg/ml) was added after each well was extensively washed with PBST. The release of 4-methylumbelliferone was monitored fluorometrically ($\lambda_{\text{ex}} = 355 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$) by fluorescence plate reader (Titertek Fluoroskan II; MP Biomedicals, Solon, OH, USA). There was no fluorescence due to OPA–protein complexes in serum. Since newly synthesized mouse IgE is specifically detected in this assay system, OPA-modified serum protein was not detected by the ELISA.

For IgG detection, anti-mouse IgG polyclonal antibody (Invitrogen) was added after the incubation with sera. After the incubation for 1 h, streptavidin conjugated with peroxidase was added and then incubated for 1 h and washed extensively with PBST. O-phenyldiamine (0.4 mg/ml) was added, and then reaction was stopped by 2 M sulfuric acid. The absorbance was read at 490 nm, spectrophotometrically by microplate reader MR-600 (DYNATECH Laboratories Ltd., Alexandria, VA, USA).

2.6. Data analysis

All data are expressed as mean $\pm$ standard deviation. One-way and two-way ANOVA with Fisher's protected least employed to compare mean values between two groups. A *p*-value of 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Lethality of OPA administration in normal and OVA sensitized mice

Though the data was not shown, lethality of OPA was tested using male and female mice. Mice were injected with OPA on days 0 and 5 in normal and OVA sensitized mice. Fifty percent of lethal dose (LD_{50}) was OPA 36.2 mg/kg body weight in OVA-sensitized male and female mice and OPA 73.3 mg/kg body weight in normal male and female mice during 18–19 days observation.

3.2. OPA induced neutrophils infiltration into alveolar space of OVA-sensitized mice

After administration of OPA to OVA-sensitized mice on days 0 and 5, mice were exposed in the inhalation chamber with OVA aerosol for 20 min on day 18 or 19. To confirm the early phase inflammation, mice were sacrificed at 3 h after antigen challenge. Results in BAL analysis are shown in Fig. 2. Total cell numbers in BAL of the sensitized mice were slightly higher than those of normal mice. Significant increase of total cell number in BAL fluid was observed in the 0.25% OPA + OVA group as compared with those of the OVA-sensitized mice without OPA.

Differential cytology indicates that the percentage of neutrophil increased significantly in the OVA-sensitized mice with OPA administration ($>0.0125\%$ of OPA), but there was no dose response with respect to OPA concentration. In contrast, the ratio of macrophages in cellularity of BAL was significantly decreased in this group of animals. There was no change in the ratio of neutrophils over macrophages in the group of animals treated with OPA without OVA. The lymphocyte population in the BAL fluid did not change with OPA treatment in both OVA sensitized or non-sensitized mice. Eosinophil was not detected in all BAL fluid tested. As not shown in the data, the total protein concentration in BAL fluid did not change in all treatment groups compared with the normal group.

Gene expression of chemokines associated with inflammation in the lung tissue of OPA-administrated mice was analyzed by RT-PCR (Fig. 3). Eotaxin mRNA was expressed in the OPA + OVA groups (OPA 0.25%; 2 of 3 mice, OPA 0.125%; 1 of 2 mice, OPA 0.025%; 1 of 2 mice) as well as the OVA + Alum group, however, this gene was not expressed in the OPA, OVA, and PBS groups. Monocyte chemotactic protein-1 (MCP-1) was expressed in the 0.25% OPA + OVA group (3 of 3 mice). Expression of MCP-1 mRNA was also observed in the 0.25% OPA alone group (1 of 3 mice), and tumor necrosis factor- α (TNF- α) was expressed only in the 0.25% OPA + OVA (2 of 3 mice). Interleukin-1 β (IL-1 β) was not expressed in all groups (data not shown).

3.3. OPA elicited allergen-specific IgE but not IgG in OVA-sensitized mice

To elucidate the effect of OPA as an IgE specific immunological adjuvant, concentration of allergen-specific IgE and IgG

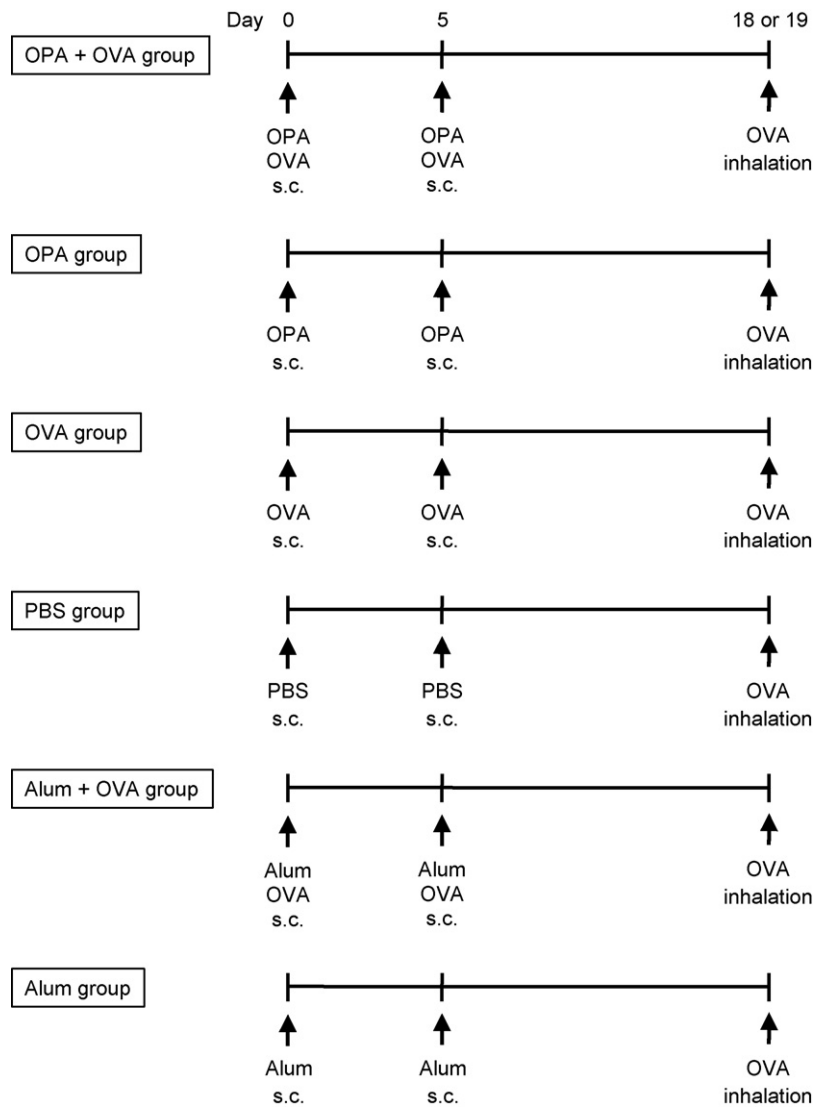


Fig. 1. Scheme of experimental protocol. OPA + OVA group: various concentration of OPA and OVA (10 μ g) were injected subcutaneously (s.c.) on day 0 and day 5. OPA group: various concentration of OPA was injected s.c. on day 0 and day 5. OVA group: OVA (10 μ g) was injected s.c. on day 0 and day 5. PBS group: PBS (200 μ l) was injected s.c. on day 0 and day 5. Alum + OVA group: Alum (4 mg) and OVA (10 μ g) were injected s.c. on day 0 and day 5. Alum group: Alum (4 mg) was injected s.c. on day 0 and day 5. All mice were then exposed to OVA vapor on day 18 or day 19.

was evaluated by ELISA method. As shown in Fig. 4, serum OVA-specific IgE was elicited by administration of OPA simultaneously with OVA when compared with normal mice administrated with various concentration of OPA. OPA enhanced OVA-specific IgE production dose-dependently. The level of OVA-specific IgE elicited by OPA was equal to one elicited by well-known adjuvant Alum, when OPA was administrated more than 0.125% into mice. In contrast, OPA had no effect on OVA-specific IgG production, although Alum enhanced OVA-specific IgG production prominently.

It has been reported that Th2 cytokines play a key role in IgE production (Bacharier and Geha, 2000), hence we examined the gene expression of Th2 cytokines in OPA-administrated mouse lung tissues. The results presented in Fig. 5 show that IL-4 was expressed in 3 of 3 in the 0.25% OPA + OVA group, and 2 of 3 in the 0.25% OPA group. IL-5 was also expressed in the OPA + OVA groups (OPA 0.25%; 3 of 3 mice, OPA 0.125%; 1 of 2 mice, OPA 0.025%; 2 of 2 mice), however IL-6 was expressed in only a few mice in the OPA + OVA groups. Th1-derived cytokine IFN- γ was not expressed in all groups.

4. Discussion

The results of this study indicate that OPA induced neutrophil infiltration into bronchoalveolar with increased production of allergen-specific IgE, suggesting the role of OPA as an immunological adjuvant. OPA has been used for high-level disinfectant as safer alternative to GTA. It has been reported that median lethal dose (LD_{50}) of GTA on subcutaneous injection into male mice was 1430 mg/kg (Uemitsu et al., 1976). In contrast, LD_{50} of OPA on subcutaneous injection into male mice was 51.1 mg/kg in our study (unpublished data). Hence OPA seems to be more toxic than GTA. Treatment of OVA-sensitized mice with OPA showed higher lethality compared with non-sensitized mice, suggesting that OPA is more lethal in allergen-sensitized mice.

Total cell number in BAL fluid was significantly increased by treatment of OVA sensitized animals with 0.25% OPA. Differential cytology also showed increased ratio of neutrophil in BAL of animals in the 0.0125% OPA + OVA group. However, there was no eosinophil infiltration in BAL of these animals. This is a much lower OPA concentration than that triggered increase of the total cell number in

Table 1
Oligonucleotide primer sequences.

Gene		Primer sequence	Product size (bp)	Annealing temperature (°C)	Cycles
Eotaxin	Sense	5'-gatgcaccctgaaagccatagtc-3'	175	57.0	30
	Antisense	5'-atcaagcagttcttaggctctgg-3'			
MCP-1	Sense	5'-tcacctgctgctactcattcacca-3'	250	59.0	30
	Antisense	5'-aaaggtgctgaagaccctaggga-3'			
TNF- α	Sense	5'-agtgggtgccagccgatgggtgt-3'	253	60.0	30
	Antisense	5'-gctgagttggctccccttctccag-3'			
IL-1 β	Sense	5'-gagcttcaggcaggcagtagtc-3'	382	53.0	35
	Antisense	5'-gtatagattcttcttggaggc-3'			
IL-4	Sense	5'-aggagaaggagcgcacatgcacga-3'	197	53.0	35
	Antisense	5'-atcgaaaagcccgaagagctctg-3'			
IL-5	Sense	5'-gagcacagtgtgaaagagacatt-3'	200	53.0	35
	Antisense	5'-atgacagggtttggaatagcattt-3'			
IL-6	Sense	5'-ttccatcagttgcttcttgg-3'	360	57.0	35
	Antisense	5'-cttcattgtactcaggtag-3'			
IFN- γ	Sense	5'-tgaacgctacacactgcac-3'	401	55.0	35
	Antisense	5'-gagctcattgaatgcttggc-3'			
GAPDH	Sense	5'-ctccactcacggcaattcaa-3'	551	54.0	25
	Antisense	5'-acggccatcacgccacagc-3'			

BAL fluid. OPA associated changes of total cell number and the cell differential cytology in the sensitized animals were more prominent than that associated with the well-known adjuvant, Alum. At the same time, chemokines, eotaxin and MCP-1, were expressed

in mice treated with OPA in OVA-sensitized mice, however TNF- α or IL-1 β were not expressed. It has been reported that IL-4 is a potent inducer of eotaxin and MCP-1 (Li et al., 1999), though the expression of eotaxin and MCP-1 well reveals to be correlate to the

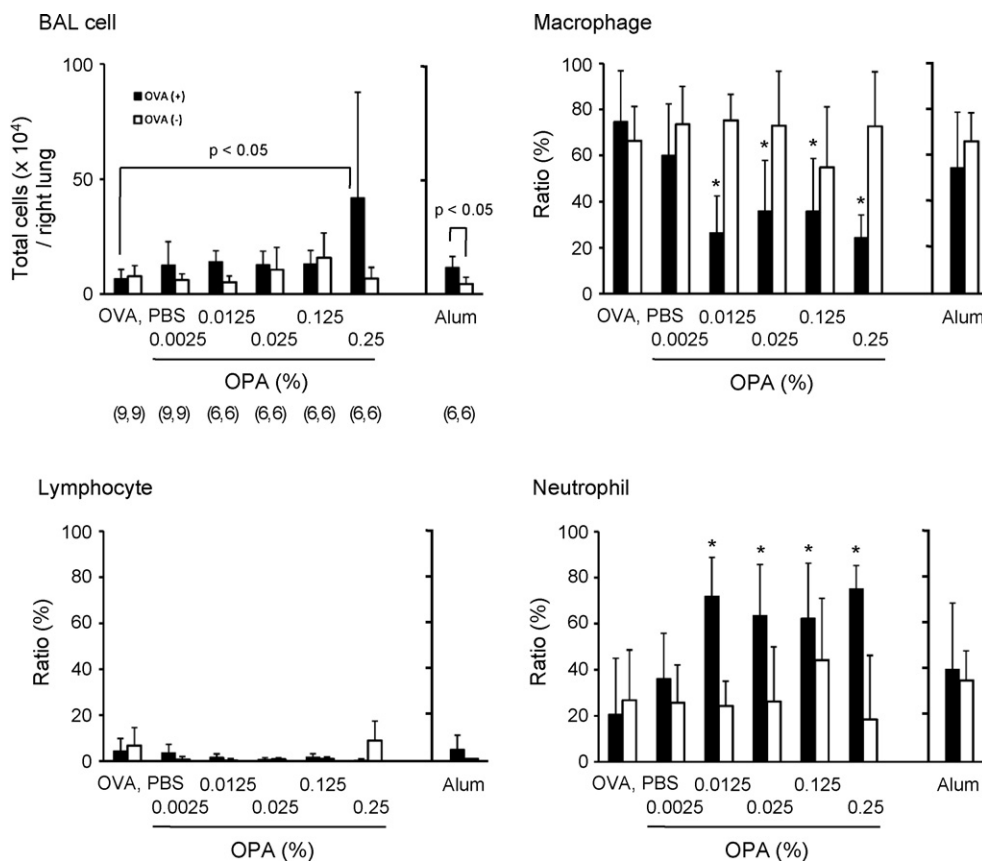


Fig. 2. The effects of OPA on total cell number and cell differentiation in BAL fluid. OPA were injected subcutaneously into mice with (solid) or without (open) OVA. Total number and the ratio of macrophage, lymphocyte and neutrophil of BAL cells were counted according to the procedure described in Section 2. Number of mice is indicated under each OPA concentration in the data of BAL cell number. The number of mice in the data of macrophage, lymphocyte and neutrophil ratio is the same as in the data of BAL cell number. The data are mean $\pm$ SD. The variation in reference to the concentration of OPA was analyzed by one-way ANOVA (BAL cell; $p=0.0221$ (OPA + OVA and OVA group), $p=0.0569$ (OPA and PBS group), macrophage; $p=0.0001$ (OPA + OVA and OVA group), $p=0.2991$ (OPA and PBS group), lymphocyte; $p=0.1173$ (OPA + OVA and OVA group), $p=0.0864$ (OPA and PBS group), neutrophil; $p<0.0001$ (OPA + OVA and OVA group), $p=0.4975$ (OPA and PBS group)). * $p<0.05$ (Ryan's method, vs OVA group).

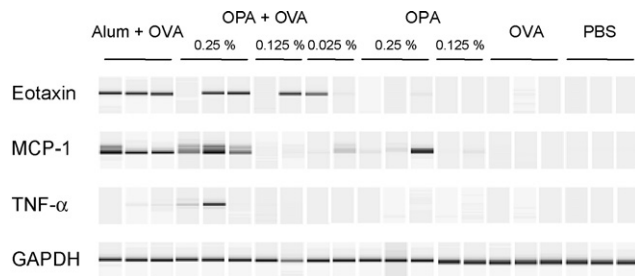


Fig. 3. Expression of chemokines in OPA-administrated mouse lung tissues. Total RNA was extracted from lung tissues of each mouse. RT-PCR was performed using specific primers for various chemokines as described in Table 1. The data shown are representative of all animals in a particular group.

expression of IL-4 in the high concentration of OPA groups. On the other hand, total BAL protein concentration did not increase significantly in all of groups. Though OPA-induced (0.0025–0.25%) and chemokine associated increase of neutrophils appeared to trigger pulmonary inflammation, the effect was transient and caused slight pulmonary injury in the present study.

Our results also suggest that OPA accelerates the allergen-specific IgE-antibody production. OPA treatment along with OVA did elicit OVA-specific IgE in the dose-dependent manner as shown in Fig. 4. OPA caused significant increase of OVA-specific IgE production at a very low dose *in vivo*. This evidence suggests that OPA has much higher efficacy as immunological adjuvant for inducing allergen-specific IgE as compared with Alum. However, the OPA did not elicit OVA-specific IgG in contrast to Alum which stimulated the OVA-specific IgG production. It is well known that Alum induces OVA-specific IgG₁ with simultaneous immunization of OVA (Faquim-Mauro and Macedo, 2000). The production of a small amount of serum anti-OVA IgG was observed by immunization of OVA without OPA. As shown in Fig. 4, the value of A_{490} of OVA group was 4.5 times higher than PBS control group (OVA group, $A_{490} = 0.009 \pm 0.007$; PBS group, $A_{490} = 0.002 \pm 0.001$). We also confirmed the production of serum anti-OVA IgG in OVA group by dot blot analysis (data not shown) which indicated much production of anti-OVA IgG. In addition, Chang et al. (2005) reported that there was little production of serum anti-OVA IgG when OVA was immunized to the animals without adjuvant. Thus we conclude though at very low level, that immune reaction was elicited by OVA without OPA in our experiments.

OVA and Alum up-regulated the gene expression of IL-4 and IL-5 in mouse lung as shown in Fig. 5. IL-4 is crucial for IgE expression, as it demonstrates pleiotropic effects which include increasing surface CD23 (FcεRII), major histocompatibility complex class II and IL-4 receptor on B cells, and inducing B cell proliferation and IgE synthesis (Bacharier and Geha, 2000). IL-5 has modulatory effects on IgE production, which include inducing isotype switching to IgE. Since OPA induced the expression of IL-4 and IL-5 and it had negligible effect on the expression of TNF-α, IL-1β and IFN-γ, the potential role of OPA is to enhance induction of Th2-type responses predominantly as compare to that of Th1-type responses. Induction of IL-4 and IL-5 appear to be specific to OPA compared with other adjuvants. Alum up-regulated IL-5 expression, while extent of expression of IL-4, IL-6 and IFN-γ was not altered (Alkan et al., 1996). On the other hand, complete Freund's adjuvant enhanced the expression of IL-6, IL-12, IFN-γ and TNF-α, while expression of IL-4 and IL-5 was not up-regulated (Billiau and Matthys, 2001). Thus we conclude that up-regulation of IL-4 and IL-5 was specific to OPA.

As shown in Fig. 4, mechanisms of adjuvant effect of OPA and Alum on Ig production are different. OPA elicited OVA-specific IgE production without increasing of OVA-specific IgG. However Alum elicited both OVA-specific IgE and IgG. Rose Bengal, which is an

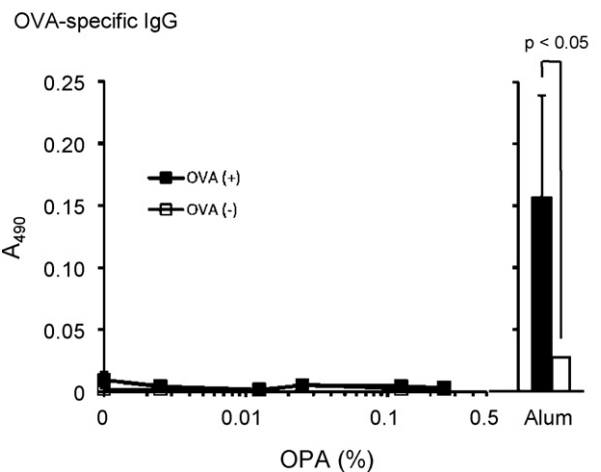
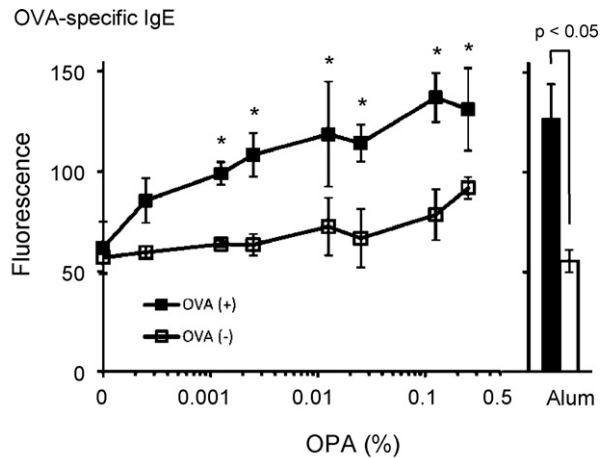


Fig. 4. The concentration of OVA-specific IgE and OVA-specific IgG. OPA were injected subcutaneously into mice with (solid) or without (open) OVA. Serum OVA-specific IgE and IgG concentrations were measured by ELISA. The data are mean $\pm$ SD ($n = 6-12$). The variation in reference to the concentration of OPA was analyzed by two-way ANOVA (OVA-specific IgE; $p < 0.0001$ (OPA), $p < 0.0001$ (OVA), $p < 0.0001$ (OPA $\times$ OVA), OVA-specific IgG; $p = 0.0058$ (OPA), $p = 0.0025$ (OVA), $p = 0.0325$ (OPA $\times$ OVA)) and one-way ANOVA (OVA-specific IgE; $p < 0.0001$ (OPA + OVA and OVA group), $p < 0.0001$ (OPA and PBS group), OVA-specific IgG; $p = 0.0182$ (OPA + OVA and OVA group), $p = 0.0383$ (OPA and PBS group)). * $p < 0.05$ (Ryan's method, vs OVA group).

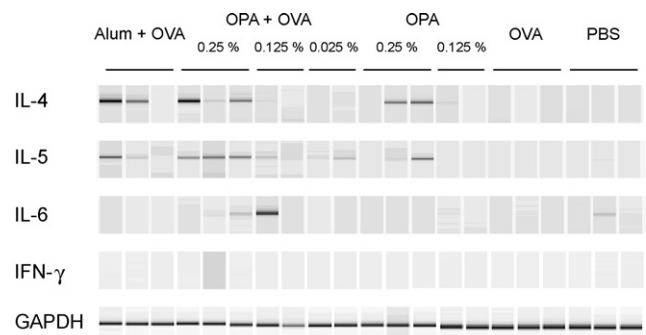


Fig. 5. Expression of Th1 and Th2 cytokines in OPA-administrated mouse lung tissues. Total RNA was extracted from lung tissues of each mouse. RT-PCR was performed using specific primers for various cytokines as described in Table 1. The data shown are representative of all animals in a particular group.

aromatic compound like OPA used for food additive, increased production of IgE, while it decreased both of IgG and IgM production in mouse B lymphoma WEHI-279 cells (Kuramoto et al., 1998). Rose Bengal enhances expression of productive ϵ ($P\epsilon$) and/or induces the synthesis of IgE, although it does not enhance the expression of germ-line ϵ transcripts that are induced prior to class-switch recombination. In other reports, CD40 signaling to B cells and T cells leads isotype switching and deletional switch recombination on Ig genes (Goedert et al., 2000; Bacharier and Geha, 2000). Taking these reports into consideration, OPA may elicit IgE production directly by enhancing $P\epsilon$ expression in B cells or indirectly by modulating CD40 signaling. Detail mechanism of OPA on IgE production will be investigated further.

Although the present study reveals that OPA caused neutrophil infiltration in bronchoalveolar, and elicited allergen-specific IgE response with very low dose concentration in the sensitized mice. The concentration of OPA disinfectant solution commercially available is 0.55% (CIDEX OPA, Johnson & Johnson, USA). By using this product, some serious cases of health effects in medical workers and patients have been reported including chemical burn injury (Venticinque et al., 2003), anaphylaxis (Sokol, 2004; Suzukawa et al., 2006, 2007), occupational bronchial asthma and contact dermatitis (Fujita et al., 2006). Our data shows that OPA induced infiltration of neutrophils followed by airway inflammation at the concentration of 0.0125%, ten times less concentration of OPA that induced allergen-specific IgE, *in vivo*. The OPA concentration which induced IgE production was much lower than that caused pulmonary injury, as shown in Figs. 2 and 4.

We conclude that OPA enhanced infiltration of inflammatory cells and increased production of allergen-specific IgE, suggesting that OPA acts as an immunological adjuvant. Therefore, as precaution for safe handling of OPA in the work place, it is important to protect medical workers and patients from dermal exposure and inhalation of OPA aerosol when it is used for sterilization.

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