

Suppression of Specific IgE Antibody Responses by Liposome-Conjugated Ovalbumin in Mice Sensitized with Ovalbumin via the Respiratory Tract

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Key Words

Suppression · Allergy · IgE · Ovalbumin ·
Liposome-conjugated antigen · Cholera-toxin-combined
antigen · Intranasal administration

Abstract

Background: Previously we have shown that intranasal administration of ovalbumin (OVA) together with cholera toxin (CT) abrogates nasal tolerance to OVA, resulting in the induction of specific IgE antibody (Ab) responses, and that intraperitoneal injection of OVA coupled with liposomes (OVA-liposomes) induces a selective suppression of IgE Ab responses to OVA. Whether OVA-liposomes suppress anti-OVA IgE Ab responses in mice sensitized with CT-combined OVA via the respiratory tract remains to be clarified. **Methods:** In some experiments, mice were given OVA, liposomes or OVA-liposomes with or without CT intranasally three times, at 2-week intervals (weeks 0, 2 and 4). In other experiments, mice were given OVA-liposomes intranasally 2 days before or 1 and

3 weeks after CT-combined OVA (week 0), which was administered intranasally three times, at 2-week intervals (weeks 0, 2 and 4). Two weeks after the third administration of CT-combined OVA (week 0), nasal wash and serum IgA, IgG and IgE Ab responses were assayed. **Results:** Pretreatment with OVA-liposomes suppressed IgE Ab responses to CT-combined OVA, with a significantly high production of both nasal IgA and serum IgG Abs. Moreover, treatment with OVA-liposomes 1 and 3 weeks after CT-combined OVA administration also suppressed IgE Ab responses. The suppression of anti-OVA IgE Ab production by OVA-liposomes was accompanied by a simultaneous enhancement of specific IgA and IgG (IgG1, and especially IgG2a) Ab production. **Conclusions:** Postimmunization treatment with OVA-liposomes, as well as preimmunization treatment, suppressed specific IgE Ab responses in mice sensitized intranasally with CT-combined OVA. Allergens conjugated to liposomes may be appropriate for preventing the development of allergies to inhaled or dietary antigens in humans.

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Introduction

Inhaled or dietary antigens, ingested via the respiratory or digestive tract mucosa, usually induce a state of specific immunological unresponsiveness that we call oral or nasal tolerance [1, 2]. However, allergies to inhaled or dietary antigens are sometimes induced by the breakdown of oral or nasal tolerance. In this regard, the nasal or oral tolerance to some antigens can be abrogated by the intranasal or oral administration of antigen together with cholera toxin (CT) and/or cholera toxin B subunit (CTB) [3–5]. Previously, we have shown that the intranasal administration of ovalbumin (OVA) together with CT abrogates nasal tolerance to OVA, resulting in an augmentation of anti-OVA IgE antibody (Ab) production [4]. These findings suggest that mice immunized intranasally with CT-combined OVA could be used as an animal model for human allergy. We have also shown that OVA coupled with liposomes (OVA-liposomes) induces selective suppression of anti-OVA IgE Ab production in BALB/c mice, with a significantly high production of IgG Ab, when injected intraperitoneally [6]. However, whether OVA-liposomes, given intranasally to mice, suppress anti-OVA IgE Ab responses in mice sensitized via the respiratory tract remains to be clarified. Intranasal immunization with OVA-liposomes seems to be a milder treatment than intraperitoneal injection. These studies may provide clues for the prevention of human allergies.

In the present study, the effects of treatment with OVA-liposomes on anti-OVA IgE Ab responses were examined in BALB/c mice immunized intranasally with CT-combined OVA. The findings showed that treatment with OVA-liposomes both before and during immunization suppressed anti-OVA IgE Ab production, with enhanced production of both nasal anti-OVA IgA and serum anti-OVA IgG Ab. The findings also showed that the ability of OVA-liposomes to suppress anti-OVA IgE production is correlated with enhanced production of IgG2a Ab. In light of these findings, we discuss mechanisms by which OVA-liposomes selectively suppress specific IgE Ab responses and the possibility of preventing the development of allergy by the nasal administration of allergen conjugated to liposomes.

Materials and Methods

Mice

Female BALB/c mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu-shi, Japan) aged 6–8 weeks at the onset of immunization were used in all experiments.

Preparation of OVA- and Bovine Serum Albumin-Liposomes

OVA was coupled with liposomes in buffer containing 0.88 mM KH_2PO_4 , 0.12 mM Na_2HPO_4 and 0.25 M saccharose, in the same manner as described previously [6]. Briefly, OVA solution (12 mg/ml, 1 ml; Sigma, St. Louis, Mo., USA) was added to a liposome solution (47.4 mg lipid/ml, 4 ml) which was composed of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidyl ethanolamine, cholesterol and palmitic acid in the molar ratio 4:3:7:2. Then, 2.4% glutaraldehyde (1 ml; Wako Purechemical Industries, Osaka, Japan) was added dropwise to the mixture. After mixing gently in a water bath at 37°C for 1 h, 2 M glycine-NaOH (pH 7.2; 1 ml) was added to block excess aldehyde groups, and the resulting mixture was kept overnight at 4°C. Uncoupled OVA or bovine serum albumin (BSA) was removed from the OVA-liposome solution by Sepharose CL-4B column chromatography (Pharmacia Biotech, Uppsala, Sweden). BSA (Sigma) coupled with liposomes (BSA-liposomes) was prepared using the same method as described above.

Immunization

Groups of five mice were anesthetized by intraperitoneal injection of amobarbital sodium (0.25 ml, 1 µg/ml) [7]. Then they were immunized intranasally with CT-combined OVA solution containing OVA (10 µg) and CT (2 µg; Sigma) in phosphate-buffered saline (PBS; 10 µl) in weeks 0, 2 and 4. Some groups of mice were given OVA-liposomes (4 µg OVA coupled with 70 µg of liposomes in 7.5 µl of PBS) intranasally 2 days before the first CT-combined OVA immunization (in week 0). Other groups of mice were given OVA-liposomes intranasally 1 and 3 weeks after the first CT-combined OVA immunization (in weeks 1 and 3). Two weeks after the third administration of CT-combined OVA, IgA, IgG and IgE Ab responses in nasal wash and serum were assayed.

Specimens

Two weeks after the third immunization, the mice were killed with chloroform and blood was obtained from the heart with a syringe. Serum samples were separated from the blood by centrifugation. After bleeding, the head of the mouse was removed and the lower jaw excised. A hypodermic needle was inserted into the posterior opening of the nasopharynx and 1 ml of PBS containing 0.1% BSA was injected three times to collect the nasal wash. The nasal wash specimens were centrifuged to remove cellular debris and used for Ab assay [7].

IgA and IgG Ab Assays

IgA and IgG Abs against OVA were determined by ELISA, as described previously [7]. Briefly, ELISA was performed sequentially from the solid phase (EIA plate; Costar, Cambridge, Mass., USA) with reagents consisting of: firstly, OVA; secondly, serial twofold dilutions of sera from each group of immunized or preimmunization mice; thirdly, goat anti-mouse IgA (α -chain specific; Southern Biotechnology Associates) or goat anti-mouse IgG Ab (γ -chain specific; Amersham) conjugated with biotin; fourthly, streptavidine conjugated with alkaline phosphatase (Gibco BRL, Paisley, Scotland), and finally, *p*-nitrophenyl-phosphate. The chromogen produced was measured for absorbance at 410 nm in an SJ EIA Autoreader (model er-8000; Sanko Junyaku Co. Ltd., Tokyo, Japan). Ab-positive cut-off values were set as mean $\pm$ 2SD of preimmune sera. ELISA Ab titer was expressed as the highest serum dilution giving a positive reaction.

Table 1. Anti-OVA Ab responses in mice immunized intranasally with CT-combined OVA-liposomes

Immunization	Anti-OVA Ab (ELISA) titer (2 ⁿ) ¹			PCA titer (2 ⁿ) ²
	serum IgG	nasal wash IgA	serum IgE	
OVA	<3.0	<1.0	<1.0	<1
OVA-lip	<3.0	<1.0	<1.0	<1
OVA/CT	7.6 ± 1.3	1.4 ± 1.1	6.4 ± 2.1	8
OVA-lip/CT	15.6 ± 1.1*	6.6 ± 1.8*	<1.0*	<1

Mice were immunized intranasally with CT-combined OVA-liposomes containing OVA-liposomes (4 µg) and CT (2 µg) in PBS (10 µl) in weeks 0, 2 and 4. Control mice were given OVA (4 µg), OVA-liposomes (4 µg) or CT (2 µg)-combined OVA (4 µg). Two weeks after the third immunization (in week 6), anti-OVA IgA, IgG and IgE Ab responses were assayed by ELISA. The serum IgE Ab titer was also analyzed by PCA against OVA. * $p < 0.05$ comparing CT-combined OVA and CT-combined OVA-liposomes.

¹ Values represent mean ± SD of Ab titers measured by ELISA in each group of five mice.

² Values represent mean Ab titer measured by PCA using pooled sera from five mice.

Titers of IgG subclass Abs to OVA were also determined by ELISA, as described above. Briefly, ELISA was performed sequentially from the solid phase with reagents consisting of: firstly, OVA molecules; secondly, twofold serial dilutions of sera; thirdly, rat anti-mouse IgG1 or IgG2a Abs conjugated with biotin (Pharmingen); fourthly, streptavidine alkaline phosphatase, and finally, *p*-nitrophenyl-phosphate. ELISA Ab titer was determined as described above.

IgE Ab Assays

IgE Ab against OVA was titrated by passive cutaneous anaphylaxis (PCA) reaction in Sprague Dawley rats (about 300 g; Shizuoka Agricultural Cooperative Association for Laboratory Animals) [8, 9]. Twofold dilutions (beginning at 1:10) of mouse sera (0.1 ml) were injected intracutaneously into the shaved back of the rats for passive sensitization. After 2 h, OVA (5 mg/ml) dissolved in PBS containing 1% Evans blue dye was injected intravenously into the rats to elicit PCA reaction. The animals were sacrificed 10 min later and the last dilution resulting in a blue-stained diameter of more than 5 mm was determined as the end-point titer.

IgE Ab against OVA was also titrated by a fluorometric reverse (IgE capture) ELISA by the method of Sakaguchi et al. [10]. Briefly, ELISA was performed sequentially from the solid phase (Dynatech Immulon 4 plates; Dynatech, Chantilly, Va., USA) with reagents consisting of: anti-mouse IgE monoclonal Ab (Yamasa Co., Chiba, Japan); PBS plus 1% BSA; twofold serial dilutions of serum; biotinylated OVA; β-*D*-galactosidase-conjugated streptavidine (Gibco) and 4-methyl-umbelliferyl-β-*D*-galactoside (Sigma), in that order. The enzyme reaction was stopped with 0.1 M glycine-NaOH (pH 10.3) and the fluorescence intensity was read on a microplate fluorescence reader. Ab-positive cutoff values were set as twice the mean fluorescence units of preimmune sera. ELISA Ab titer was expressed as the highest serum dilution giving a positive reaction.

Statistics

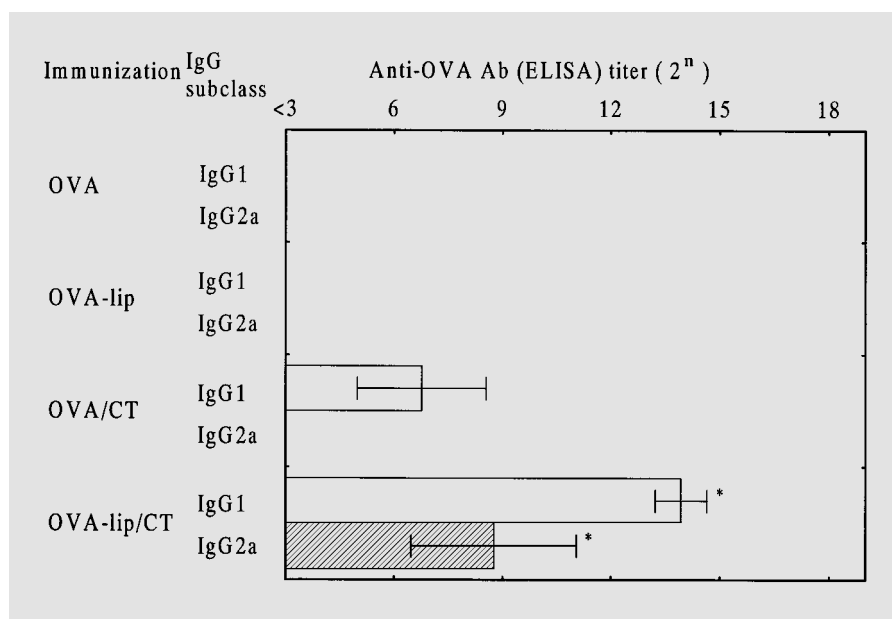
Comparisons between experimental groups were performed using Student's *t* test. $p < 0.05$ was considered significant.

Results

IgE Ab Responses in Mice Immunized Intranasally with CT-Combined OVA-Liposomes

The effects of intranasal administration of OVA-liposomes together with CT on IgE, IgA and IgG Ab responses to OVA were examined in mice immunized intranasally with CT-combined OVA-liposomes in weeks 0, 2 and 6. Two weeks after the third immunization (week 6), anti-OVA Ab responses were assayed by ELISA for IgA and IgG Ab, and by both ELISA and PCA for IgE Ab. Table 1 shows that control mice given OVA or OVA-liposomes intranasally failed to produce any isotype of Ab against OVA. Another control group of mice given CT-combined OVA intranasally produced a high level of serum anti-OVA IgE Ab, as well as nasal wash anti-OVA IgA Ab and serum anti-OVA IgG Ab. Thus, it was shown that CT abrogates the unresponsiveness to OVA when administered intranasally to mice together with OVA [4]. On the other hand, CT-combined OVA-liposomes completely suppressed IgE Ab responses and significantly enhanced IgA and IgG Ab responses. The results for IgE Ab responses obtained by ELISA assay were supported by those from PCA assay. In addition, figure 1 shows that CT-combined OVA-liposomes induced anti-OVA IgG2a Ab production with enhanced anti-OVA IgG1 Ab production, while CT-combined OVA only induced anti-OVA IgG1 Ab production, and to a lesser extent. Thus, the findings that intranasal administration of CT-combined OVA-liposomes induces anti-OVA IgA and IgG Ab production, but not anti-OVA IgE Ab production, are consis-

Fig. 1. OVA-specific IgG subclass Ab responses in the sera of mice immunized intranasally with various OVA antigens 3 times, at 2-week intervals. Each column represents the mean ($\pm$ SD) Ab titer of five mice in each group. * Significant difference from one of the controls (OVA/CT).



tent with our previous data that intraperitoneal injection of OVA-liposomes induces anti-OVA IgG Ab production with selective suppression of IgE Ab production [6]. This suggests that OVA-liposomes have the ability to suppress anti-OVA IgE Ab responses while enhancing anti-OVA IgA and IgG (IgG1 and IgG2a) Ab responses, regardless of the administration route.

Effects of Preimmunization Treatment with OVA-Liposomes on IgE Ab Responses

The effects of pretreatment with OVA-liposomes on anti-OVA IgE Ab responses were examined in mice immunized intranasally with CT-combined OVA. The mice were given OVA-liposomes intranasally, and 2 days later were immunized intranasally with CT-combined OVA, which was given three times in total, at 2-week intervals. As controls, other mice were pretreated with PBS or OVA (4 μ g), empty liposome and BSA-liposome. Two weeks after the third administration of CT-combined OVA, nasal wash IgA Ab and serum IgG and IgE Ab responses were assayed by ELISA. Table 2 shows that pretreatment with OVA-liposomes significantly suppressed anti-OVA IgE Ab responses while enhancing anti-OVA IgA and IgG Ab responses. On the other hand, PBS, OVA, empty liposome and BSA-liposome failed to suppress anti-OVA IgE Ab responses. Thus, pretreatment with OVA-liposomes induced an antigen-specific selective suppression of IgE Ab responses. Pretreatment with OVA-liposomes 1 week before immunization also selectively suppressed IgE Ab

Table 2. Anti-OVA Ab responses in mice treated with OVA-liposomes before immunization

Treatment	Anti-OVA Ab (ELISA) titer (2^n)		
	serum IgG	nasal wash IgA	serum IgE
Exp. 1			
PBS (-)	7.8 $\pm$ 0.4	3.0 $\pm$ 0.7	5.2 $\pm$ 1.5
OVA	9.8 $\pm$ 1.9	3.8 $\pm$ 0.8	6.0 $\pm$ 1.6
OVA-lip	17.2 $\pm$ 2.9 ^{1,2}	5.0 $\pm$ 1.6 ¹	2.2 $\pm$ 2.3 ^{1,2}
Exp. 2			
PBS (-)	11.0 $\pm$ 1.7	NT	5.5 $\pm$ 0.6
Empty lip	9.3 $\pm$ 1.3	NT	6.3 $\pm$ 1.2
BSA-lip	11.3 $\pm$ 2.2	NT	6.3 $\pm$ 1.5
OVA	13.3 $\pm$ 3.1	NT	7.7 $\pm$ 1.5
OVA-lip	16.8 $\pm$ 0.8 ¹⁻⁴	NT	2.0 $\pm$ 2.4 ¹⁻⁴

Mice were given OVA-liposomes intranasally two days before the first immunization with CT-combined OVA. CT-combined OVA was given three times at 2-week intervals. Two weeks after the third administration of CT-combined OVA, anti-OVA IgG, IgA and IgE Ab responses were assayed by ELISA. Each value represents the mean $\pm$ SD of Ab titers of the five mice in each group. NT = Not tested.

¹ Significant difference between PBS (-) and OVA-liposomes ($p < 0.05$).

² Significant difference between OVA and OVA-liposomes ($p < 0.05$).

³ Significant difference between empty liposomes and OVA-liposomes ($p < 0.05$).

⁴ Significant difference between BSA-liposomes and OVA-liposomes ($p < 0.05$).

Fig. 2. OVA-specific IgG subclass Ab responses in the sera of mice treated with OVA-liposomes 2 days before immunization. Mice were immunized intranasally with CT-combined OVA 3 times, at 2-week intervals. Each column represents the mean ($\pm$ SD) Ab titer of five mice in each group. ¹ Significant difference between PBS (-) and OVA-liposomes ($p < 0.05$). ² Significant difference between OVA and OVA-liposomes ($p < 0.05$).

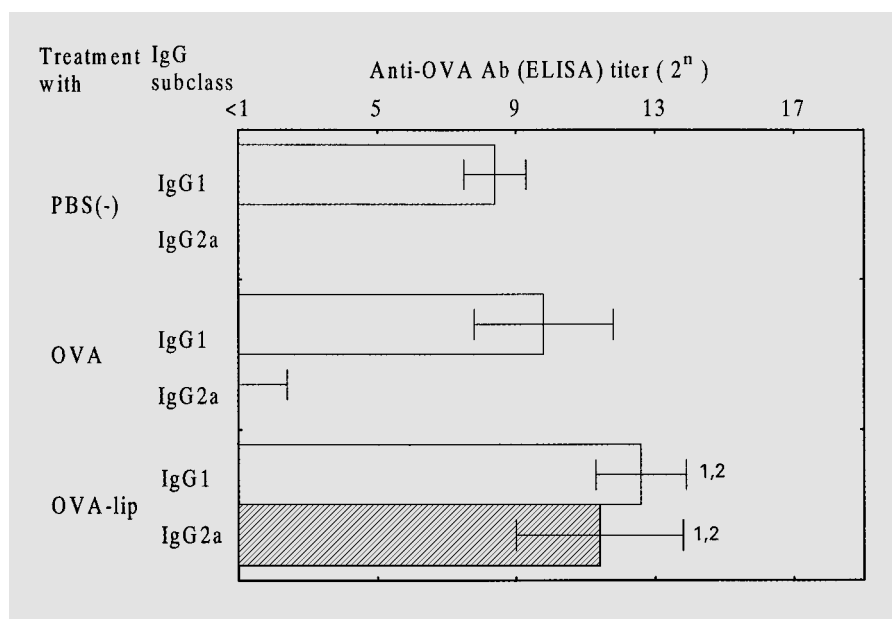
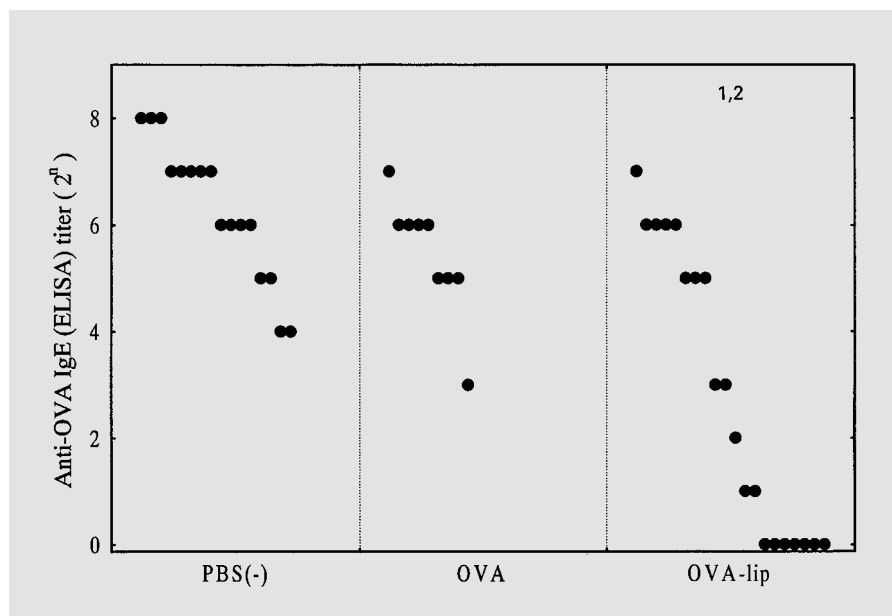


Fig. 3. Effects of postimmunization treatment with OVA-liposomes on OVA-specific IgE Ab responses. Mice were immunized intranasally with CT-combined OVA 3 times, at 2-week intervals (in weeks 0, 2 and 4) and treated with OVA-liposomes in weeks 1 and 3 after immunization. Each dot represents the IgE Ab titer, measured by ELISA, of each mouse in each group. Mean ($\pm$ SD) Ab titers of the group that received treatment with PBS, OVA and OVA-liposomes during immunization were 6.3 ± 1.3 , 5.4 ± 1.1 and 2.8 ± 2.7 , respectively. ¹ Significant difference between PBS (-) and OVA-liposomes ($p < 0.0001$). ² Significant difference between OVA and OVA-liposomes ($p < 0.01$).



production (data not shown). Moreover, figure 2 shows that treatment with OVA-liposomes induced a significantly high level of anti-OVA IgG2a Ab production, with enhanced IgG1 Ab responses. These results demonstrate that pretreatment with OVA-liposomes suppressed anti-OVA IgE Ab responses, with selective induction of anti-OVA IgG2a Ab and enhanced production of anti-OVA IgA and IgG1 Ab, in mice given CT-combined OVA intranasally.

Effects of Treatment with OVA-Liposomes during Immunization on IgE Ab Responses

The effects of treatment with OVA-liposomes during immunization on anti-OVA IgE Ab responses were examined in mice immunized intranasally with CT-combined OVA. The mice were immunized intranasally with CT-combined OVA in weeks 0, 2 and 4 (three times, at 2-week intervals) and treated intranasally with OVA-liposomes in weeks 1 and 3 (1 and 3 weeks after the first CT-combined OVA immunization). Control groups of mice

Fig. 4. OVA-specific IgG subclass Ab responses in the sera of mice treated with OVA-liposomes 1 and 3 weeks after intranasal immunization with CT-combined OVA. Each column represents the mean ($\pm$ SD) Ab titer of 16 mice for treatment during immunization with PBS, 9 mice with OVA and 20 mice for OVA-liposomes. ¹ Significant difference between PBS (-) and OVA-liposomes ($p < 0.05$). ² Significant difference between OVA and OVA-liposomes ($p < 0.05$).

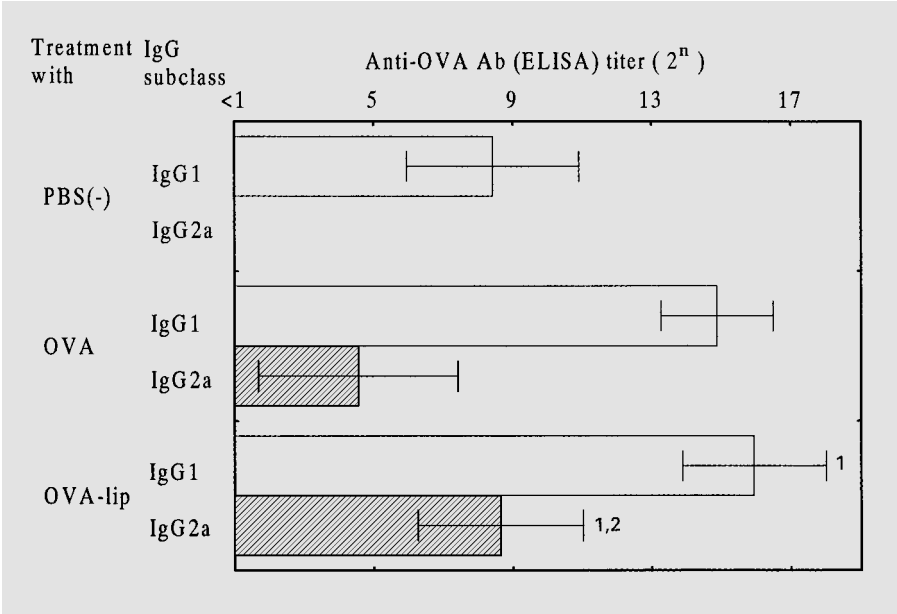


Table 3. Anti-OVA Ab responses in mice treated intranasally with OVA-liposomes during immunization with CT-combined OVA

Treatment	Anti-OVA Ab (ELISA) titer (2 ⁿ)	
	serum IgG	nasal wash IgA
PBS (-)	10.6 $\pm$ 2.0	0.3 $\pm$ 0.5
OVA	18.3 $\pm$ 2.4	1.0 $\pm$ 1.1
OVA-lip	17.5 $\pm$ 1.0 ¹	3.6 $\pm$ 1.9 ^{1,2}

Mice were immunized intranasally with CT-combined OVA in weeks 0, 2 and 4 and treated intranasally with OVA-liposomes in weeks 1 and 3 (1 and 3 weeks after the first CT-combined OVA immunization). Control groups of mice were treated with PBS or OVA, instead of OVA-liposomes. Two weeks after the third administration of CT-combined OVA, anti-OVA IgG, IgA and IgE Ab responses were assayed by ELISA. Each value represents the mean $\pm$ SD of Ab titers of 16 mice for the PBS-treated group, 9 mice for the OVA-treated group and 20 mice for the OVA-liposome-treated group.

¹ Significant difference between PBS (-) and OVA-liposomes ($p < 0.05$).

² Significant difference between OVA and OVA-liposomes ($p < 0.05$).

were treated with PBS or OVA (4 μ g), instead of OVA-liposomes. Two weeks after the third administration of CT-combined OVA, nasal wash IgA Ab and serum IgG and IgE Ab responses were assayed by ELISA. Table 3 shows that treatment with OVA-liposomes significantly

enhanced nasal wash anti-OVA IgA Ab responses, with a tendency also to enhance serum anti-OVA IgG Ab responses. On the other hand, figure 3 shows that treatment with OVA-liposomes significantly suppressed serum anti-OVA IgE Ab responses. Thus, OVA-liposomes significantly suppressed anti-OVA IgE Ab responses while enhancing anti-OVA IgA and IgG Ab responses. Moreover, figure 4 shows that treatment with OVA-liposomes induced significantly higher anti-OVA IgG2a Ab production than that with OVA, and significantly higher anti-OVA IgG1 Ab production than that with PBS. These results demonstrate that treatment with OVA-liposomes during immunization selectively suppressed anti-OVA IgE Ab responses, while enhancing the production of anti-OVA IgA and IgG Ab (IgG1 and IgG2a), in mice given CT-combined OVA intranasally.

Discussion

In the present experiments, mice immunized intranasally with CT-combined OVA three times, at 2-week intervals, were tested as a useful model for allergies via the respiratory mucosa. Preimmunization treatment with OVA-liposomes in the immunized mice selectively suppressed OVA-specific IgE Ab responses while enhancing the production of both nasal IgA and serum IgG Abs (table 2, fig. 4). Moreover, treatment with OVA-liposomes 1 and 3 weeks after CT-combined OVA immunization also

suppressed IgE Ab responses (fig. 3). Preimmunization treatment seemed to suppress the induction phase of anti-OVA IgE Ab responses, while treatment during immunization seemed to suppress the development of primed cells (early-expression phase). Thus, these findings suggest that intranasal administration of OVA-liposomes can suppress not only the induction phase, but also the early-expression phase, of anti-OVA IgE Ab responses in mice given CT-combined OVA intranasally. These findings are consistent with those of intraperitoneal immunization studies, in which OVA-liposomes suppressed specific IgE Ab responses even after a subsequent challenge with OVA adsorbed to aluminium hydroxide (OVA-alum), and following the primary immunization with OVA-alum, a secondary challenge with OVA-liposomes suppressed the secondary anti-OVA IgE Ab production [6]. Thus, OVA-liposomes have the ability to suppress not only the induction, but also the activation of primed immunocompetent cells involved in anti-OVA IgE Ab responses, regardless of the administration route.

In the present experiments, it was also shown that the ability of OVA-liposomes, given intranasally, to suppress anti-OVA IgE Ab production was correlated with a significantly enhanced production of IgG2a Ab in mice immunized intranasally with CT-combined OVA. Similar results have also been obtained in mice immunized intraperitoneally with OVA-liposomes [6]. In mice, the production of selective immunoglobulin isotypes is known to be regulated by two types of helper T cells: Th1 cells secrete IFN- γ which supports IgG2a Ab production, and Th2 cells secrete IL-4 which supports IgG1 and IgE Ab production [11]. In this regard, we have previously shown that spleen cells taken from mice immunized intraperitoneally with OVA-liposomes and OVA-alum predominantly produce IFN- γ and IL-4, respectively, with other cytokine profiles which do not necessarily agree with Th1/Th2 cytokine patterns [12]. In the present experiments, the mechanisms by which CT abrogates unresponsiveness to OVA in mice given CT-combined OVA intranasally may be explained by the production of proinflammatory cytokines (IL-1, IL-6 and IL-8) by mucosal epithelial cells and the upregulation of IL-1 production and expression of the costimulatory molecule B7.2 by macrophages or antigen-presenting cells (APC) [13, 14]. In addition, enhancement of the expression of B7.2 molecules on APC seems to result in the induction of Th2 cells and then IgG1 Ab production, as reported by Cong et al. [13]. These reports suggest that the enhancement of IgE, IgG1 or IgA Ab production in mice given CT-combined OVA intranasally (tables 1–3, fig. 1–4) is derived from the

activation of OVA-specific Th2 cells. On the other hand, OVA-liposomes which suppress IgE Ab production but enhance IgG2a Ab production in mice given CT-combined OVA (table 2, fig. 2–4) must activate Th1 cells which produce IFN- γ . Simultaneously, OVA-liposomes enhance IgG1 and IgA Ab production (table 2, fig. 2, 4), which must be mediated by IL-4. In this regard, preliminary experiments were done to examine the expression of cytokine mRNAs in nasal-associated lymphoid tissue (NALT), which has been recognized as the only well-organized mucosal-associated lymphoid tissue in the respiratory tract [15, 16], in mice given OVA-liposomes 2 days previously and immunized intranasally with CT-combined OVA three times, at 2-week intervals. The results showed that the expression of IL-4 mRNA in the isolated NALT was observed predominantly in the group pretreated with OVA-liposomes (data not shown). However, these findings could not explain the mechanisms by which OVA-liposomes suppress IgE Ab production, while enhancing the production of IgA and IgG Ab (IgG1 and IgG2a). Further studies are required to clarify unknown pathways which are specific for anti-OVA IgE Ab production but not for IgA and IgG Ab production, and in which the induction and development of IgE-specific primed cells are suppressed by OVA-liposomes.

Mucosal administration of allergens or autoantigens may be an efficient way to prevent diseases associated with untoward immune responses to these antigens, and autoimmune diseases [1, 2]. However, this approach has its limitations, because it usually requires repeated administrations of large doses of antigen, and is less efficient in previously immunized hosts. In this regard, Sun et al. [17] have shown that a small amount of sheep red blood cells or human γ -globulin coupled to CTB can markedly suppress systemic immune responses (IgM and IgG) when administered orally in naive as well as in immune animals. We have also shown that a small amount of OVA conjugated to *Escherichia coli* heat-labile toxin B subunit (LTB) can suppress subsequent delayed-type hypersensitivity and IgE Ab responses to OVA when administered intranasally [18]. Thus, intranasal or oral administration of antigens conjugated to mucosa-binding molecules, such as CTB and LTB, could inhibit subsequent IgM, IgG and IgE Ab responses and delayed-type hypersensitivity. On the other hand, in the present experiments, intranasal administration with a relatively small amount of OVA-liposomes was shown to suppress subsequent anti-OVA IgE, but not IgG and IgA Ab responses (table 2, fig. 2). Anti-OVA IgA and IgG Abs generated by OVA-liposomes may decrease the antigens which stimulate memory cells

for specific IgE production on reexposure to OVA via respiratory mucosa. Moreover, it was shown that treatment with OVA-liposomes during immunization, as well as preimmunization treatment, suppressed specific IgE Ab responses in mice sensitized intranasally with CT-combined OVA (table 2, fig. 2). These findings suggest that the mechanisms by which OVA-liposomes selectively suppress IgE Ab responses are different from the OVA-LTB immune suppression responses. The strategy of mucosal tolerance induction with CTB- or LTB-conjugated antigens may find broad application in suppressing au-

toimmune diseases, while the strategy of IgE tolerance induction with liposome-conjugated antigens may be favorable for suppressing allergies via the mucosa, or as side effects of vaccines. Thus, a prophylactic treatment with inhaled or dietary antigens conjugated to liposomes may prevent the induction of allergies caused by these antigens, and then treatment with liposome-conjugated allergens before ingesting the allergens may also suppress the further development of allergies in patients who are allergic to inhaled or dietary antigens.

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