

Effects of cholera toxin adjuvant on IgE antibody response to orally or nasally administered ovalbumin

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Effects of cholera toxin B subunits supplemented with 0.1% cholera toxin (CTB) on systemic IgE antibody responses to ovalbumin (OVA) were examined in BDF1 (H-2^{b/d}), Balb/c (H-2^d) and C3H (H-2^k) mice given OVA intragastrically or intranasally. Two successive doses of OVA intragastrically administered to Balb/c and C3H mice induced little IgE response and resulted in almost complete unresponsiveness to subsequent intraperitoneal challenge with OVA in Al(OH)₃, while the intragastric administration to BDF1 mice induced high IgE response and resulted in abrogation of the unresponsiveness to the subsequent challenge. The intranasal administration of OVA induced little IgE response and suppressed response to the subsequent challenge in any strain of mice. On the other hand, two successive doses of intragastric or intranasal OVA together with CTB* enhanced IgE response in all three strains and the subsequent challenge with OVA in Al(OH)₃ induced higher IgE responses. These results suggest that CTB* augments IgE response to OVA and abrogates the unresponsiveness when administered orally or intranasally into mice together with OVA, regardless of the H-2 haplotype of the mice.*

Keywords: Cholera toxin; IgE antibody response; oral tolerance; cholera toxin B subunit; ovalbumin

The state of specific immunological unresponsiveness, 'oral tolerance', is induced by previous oral administration of dietary protein antigens¹. The oral tolerance is most readily induced in IgE and delayed-type hypersensitivity (DTH) responses and the resulting tolerance seems to be the means to prevent hypersensitivity to orally administered antigens. In addition, the specifically suppressed IgE response is also induced by prior inhalation of antigens². On the other hand, cholera toxin (CT) or cholera toxin B subunit (CTB) containing a small amount of CT, when administered orally together with unrelated antigens, abrogates the oral tolerance to the unrelated antigens and results in augmentation of IgA and IgG responses to the antigens^{3,4}. We have also demonstrated that CTB containing a trace amount of CT (CTB*) administered orally or intranasally together with the inactivated influenza vaccine enhanced IgA and IgG responses to the vaccine⁵⁻⁷. These results suggest that the oral or intranasal administration of CTB* together with dietary protein antigens abrogates tolerance to the dietary antigens in the IgE antibody class as well as in the IgA and IgG antibody classes. In the

present experiments we examined the effects of orally or intranasally administered CTB* on the IgE responses to dietary protein, ovalbumin (OVA) in high (BDF1, H-2^{b/d}), medium (Balb/c, H-2^d) and low (CH3, H-2^k) responders to CTB* (Ref. 8).

Female mice of the respective strains (6-8 weeks old, Japan SLC Inc., Hamamatsu-shi, Japan) were immunized by gastric intubation of 150 µl OVA (Sigma Chemical Co., St Louis, MO) solution (20 mg/mouse) containing sodium bicarbonate with or without CTB* (10 µg/mouse; CTB purified by us and supplemented with 0.1% CT^{6,9}, on two occasions at 10-day intervals. Ten days after the second oral immunization the mice were challenged by intraperitoneal injection with OVA (10 µg/mouse) in Al(OH)₃ (1 mg/mouse)¹⁰. Serum samples were collected on days 10, 20 and 34 (14 days after challenge) and the IgE and IgG antibodies were titrated by passive cutaneous anaphylaxis (PCA)¹¹ and ELISA¹², respectively. Table 1 shows the results. Two successive oral doses of OVA in Balb/c and C3H mice induced little IgE response and the subsequent challenge with OVA in Al(OH)₃ suppressed the induction of IgE response to the challenge antigen 14 days after challenge. The oral immunization of BDF1 mice induced high IgE response and the subsequent challenge failed to suppress the IgE response. On the other hand, two oral doses of OVA together with CTB* induced IgE response and resulted in abrogation of the suppression to the parenteral challenge in C3H and Balb/c mice. The oral administration of CTB*-combined OVA to BDF1 mice also

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Table 1 Effects of CTB* on IgE and IgG antibody responses to OVA in various strains of mice immunized intragastrically with OVA and challenged intraperitoneally with OVA in Al(OH)₃

Mouse strain	Antigens (intragastric) ^a		OVA in Al(OH) ₃ (intraperitoneal) Day 20	IgE PCA titres ^b (IgG; ELISA units ml ⁻¹)		
	Day 0	Day 10		Day 10	Day 20	Day 34
C3H	—	—	+	—	—	320 (22 ± 11)
	OVA	OVA	+	<5	<5	<5 (975 ± 908*)
	OVA/CTB*	OVA/CTB*	+	20	160	80 (1926 ± 740*)
Balb/c	—	—	+	—	—	160 (7 ± 2)
	OVA	OVA	+	<5	5	5 (61 ± 38*)
	OVA/CTB*	OVA/CTB*	+	80	80	160 (400 ± 262*)
BDF1	—	—	+	—	—	320 (20 ± 8)
	OVA	OVA	+	320	80	160 (122 ± 29*)
	OVA/CTB*	OVA/CTB*	+	1280	80	320 (145 ± 26*)

^aMice were immunized by intragastric administration of CTB*-combined OVA on days 0 and 10, and boosted by intraperitoneal administration of OVA in Al(OH)₃ on day 20.

^bSerum samples were collected by partial bleeding on the 10th, 20th and 34th days after the first intragastric immunization and IgE antibody in the pooled sera from five mice per group was measured by PCA. Variations of the titre for the same sample in duplicate was less than twofold. Difference in the titres more than fourfold between groups was considered significant.

^cIgG antibody on the 34th day (the 14th day after challenge) was determined by ELISA (units ml⁻¹). The pooled sera from C3H mice having received intragastric administration of CTB*-combined OVA were used as a 1600 unit standard for anti-OVA IgG. Values represent means ± s.d. of the units from five mice per group. Asterisks (*) indicate a significant difference between the groups ($p < 0.05$).

Table 2 Effects of CTB* on IgE and IgG antibody responses to OVA in various strains of mice immunized intranasally with OVA and challenged intraperitoneally with OVA in Al(OH)₃

Mouse strain	Antigens (intranasal) ^a		OVA in Al(OH) ₃ (intraperitoneal) Day 20	IgE PCA titres ^b (IgG; ELISA units ml ⁻¹)		
	Day 0	Day 10		Day 10	Day 20	Day 34
C3H	—	—	+	—	—	160 (16 ± 7)
	OVA	OVA	+	<5	<5	10 (139 ± 104*)
	OVA/CTB*	OVA/CTB*	+	5	10	80 (314 ± 196*)
Balb/c	—	—	+	—	—	160 (8 ± 2)
	OVA	OVA	+	<5	<5	10 (51 ± 9*)
	OVA/CTB*	OVA/CTB*	+	10	40	160 (74 ± 16*)
BDF1	—	—	+	—	—	240 (12 ± 2)
	OVA	OVA	+	<5	5	10 (48 ± 27*)
	OVA/CTB*	OVA/CTB*	+	80	160	480 (119 ± 52*)

^aMice were immunized by intranasal administration of CTB*-combined OVA on days 0 and 10, and boosted by intraperitoneal administration of OVA in Al(OH)₃ on day 20. For other details, see footnote to Table 1.

enhanced IgE response. Thus, the oral immunization with CTB*-combined OVA induced higher IgE response than that with OVA alone before or after the challenge of OVA in Al(OH)₃ in any strain of mice.

Other groups of mice were immunized by intranasal administration of 10 µl PBS containing OVA (100 µg/mouse) with or without CTB* (2 µg/mouse), according to the time schedule described above (Table 2). Two successive intranasal doses of OVA induced little IgE response and the subsequent challenge suppressed IgE response in any strain of mouse. On the other hand, two nasal doses of OVA together with CTB* induced IgE response. The antibody responses in order of magnitude were BDF1, Balb/c and C3H. The subsequent challenge with OVA in Al(OH)₃ further enhanced the responses.

For IgG response, two oral or intranasal immunization doses of OVA and the subsequent challenge with OVA in Al(OH)₃ produced a higher level of response than the subsequent challenge alone. Oral or intranasal immunization with OVA together with CTB* and the subsequent challenge further augmented the responses (Tables 1 and 2). The IgG responses in order of magnitude were CH3 and Balb/c (or BDF1).

The results described above demonstrate that CTB* enhances the IgE response to OVA and abrogates the tolerance for the IgE responses. Moreover, they confirm that the adjuvant effect of CTB* is manifested regardless of the H-2 haplotype of the mice⁸. The oral or nasal tolerance to dietary protein antigens seems to be the means to prevent hypersensitivity^{1,2}. Therefore, the abrogation of the tolerance for IgE response to dietary antigens by CTB* may lead to allergic disease. Moreover, with respect to the IgE responsiveness to OVA, BDF1 (H-2^{b/d}), Balb/c (H-2^d) and C3H (H-2^k) were high, intermediate and low responders, respectively, whereas with respect to the IgG responsiveness, C3H and Balb/c (and BDF1) were high and low responders, respectively. Thus, an entirely different type of genetic control for IgG isotype was found in the mouse^{13,14}. The results also show dissociation of IgE response between intragastric and intranasal routes of administration in BDF1 mice, suggesting that the gut-associated lymphoid tissue stimulated by oral administration is under different control of immune responses from the nasopharyngeal lymphoid tissue stimulated by intranasal administration^{15,16}.

The mucosal immune system should provide rapid and potent immune responses to prevent invasion by pathogenic viruses, bacteria or other parasites. Stimulation of mucosal IgA production would improve the protective efficacy of the various inactivated vaccines. In this regard, we have demonstrated that intranasal or oral inoculation with influenza vaccine together with CTB* enhances IgA response to the vaccine and provides crossprotection against influenza virus infection⁵⁻⁷. The vaccine used was an ether split-product vaccine prepared from influenza viruses grown in eggs. The present experiments demonstrated that IgE response to OVA, inoculated intranasally together with CTB*, is also enhanced. These facts suggest that OVA contaminating CTB*-combined influenza vaccine would lead to food allergy. However, we failed to detect anti-OVA IgE response in Balb/c mice having received three successive doses of intranasal CTB*-combined influenza vaccine at 10-day intervals and the challenge immunization with OVA in Al(OH)₃ (data not shown). The current split-product influenza vaccine containing only a negligible amount of OVA would minimize a potential risk of allergy, even when inoculated intranasally together with CTB*.

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