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Oral Administration of T Cell Epitope Peptide Inhibits the Systemic IL-4 Response Elicited by an Egg-White Diet in a TCR Transgenic Mouse Model

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Oral immunotherapy with T cell epitope peptides is a promising treatment for food allergy. We examined the effect of oral administration of an ovalbumin T cell epitope peptide (OVA323-339) in a TCR transgenic mouse model (OVA23-3 mice). OVA23-3 mice were fed egg-white diet containing ovalbumin and subsequently orally administrated the OVA323-339 peptide. Cytokine measurements revealed that the IL-4 production of splenic $CD4^+$ T cells was significantly decreased by feeding the OVA323-339 peptide. Our study suggested that oral administration of the OVA323-339 T cell epitope peptide was capable of inhibiting systemic IL-4 response after elicitation of predominant Th2 responses.

Key words: food allergy, oral immunotherapy, ovalbumin, T cell epitope, TCR transgenic mice

In recent decades, the prevalence of food allergy has increased remarkably, particularly in developed countries. The main treatments for patients with food allergy are limited to symptomatic therapies, such as avoiding food allergens and taking medications. These therapies are a physical burden on patients and their families; therefore, establishment of a radical cure has been desired greatly. Recently, several new approaches for treatment of food allergy have been proposed and are under investigation actively. Oral immunotherapy (OIT) is a treatment in which patients are orally administrated with small amounts of a causative food of allergic symptoms. OIT with dietary foods containing natural allergens has been performed in clinical tests, but side effects of the food have been observed [1]. Natural allergens contain T and B cell epitopes. Because natural allergens have intact B cell epitopes that cross-link IgE bound to IgE receptors, side effects of IgE-mediated severe allergic symptoms are likely to be induced. To solve this problem caused by using natural allergens in OIT, T cell epitopes, which do not contain intact IgE-binding epitopes, have been proposed for safe and effective OIT. Food allergy is thought to be caused by abnormal Th2 immune responses to food allergen; in particular, $CD4^+$ T cells play the central role in different symptoms involving IgE production. Thus, ingestion of peptides corresponding to epitopes of Th2 $CD4^+$ T cells of an allergen has attracted attention as a promising approach to reduce abnormal Th2 immune responses. This approach can precisely target T cells alone and induce T cell tolerance. In fact, the expected effect of OIT using a T cell epitope has been shown in animal models of pollen allergy [2], bronchial asthma [3], and food allergy [4].

To examine the efficacy of oral administration of a T cell epitope in mice, we used a food allergy model of OVA23-3 mice. This transgenic strain of mice expresses a T cell antigen receptor (TCR) specific for the 323-339 region of a major egg allergen, ovalbumin (OVA), and its genetic background is BALB/c. We have shown previously that this mouse strain is a food allergy model exhibiting Th2 cytokine response, high levels of IgE production, weight loss and intestinal inflammation following feeding of an egg-white diet (EW diet) [5]. OVA23-3 mice show food-allergic inflammation in response to feeding of the EW diet alone; therefore, use of this mouse model may allow us to easily evaluate the efficacy of a T cell epitope peptide. The 323-339 region of OVA was reported to be a T cell epitope not only for BALB/c mice [6] but also for some food-allergic patients [7]. Therefore, the OVA323-339 peptide may be a promising candidate

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peptide for allergen-specific immunotherapy, and study using the OVA323-339 peptide has the potential to give us important information for exploiting OIT.

Although previous studies using animal models have demonstrated the efficacy of feeding T-cell epitope peptides before sensitization with an allergen, the effects after sensitization have not been well evaluated. In this study, to evaluate the capability of treatment with a T cell epitope, we administered the OVA323-339 peptide to OVA23-3 mice after induction of a Th2 response by EW-diet-feeding.

First, OVA23-3 mice (over 8 weeks of age) were divided into 2 experimental groups. The EW group was fed the EW diet (Funabashi Farm, Funabashi, Japan) [5] containing OVA for 7 days. The control group was fed CE-2 diet (Clea Japan Inc.) for 14 days. At day 7, several mice from each group were sacrificed for cell culture and cytokine measurements. As a next step, the remaining mice in the EW group were divided into two experimental groups, EW/Peptide and EW/Saline. Mice in EW/Peptide group were orally administered OVA323-339 peptide, and those in the EW/Saline group were orally administered saline; administration was on days 7, 9 and 11, respectively. At day 14, mice from each group were sacrificed for cell culture and cytokine measurements (Fig. 1). OVA23-3 mice were originally kindly provided by Drs. Sonoko Habu and Takehito Sato. We used female OVA23-3 mice in this study. The experiments were performed in accordance with guidelines for animal care and use of the University of Tokyo and approved by the Animal Use Committee of the Faculty of Agriculture, the University of Tokyo. OVA323-339 synthetic peptide (ISQAVHAAHAEINEAGR) with a purity > 75% was purchased from Biologika (Nagoya, Japan). The OVA323-339 peptide was suspended to a concentration of 2.5 mM in 200 µl saline and administrated to mice by intragastric feeding. For cell culture and cytokine measurements, splenocytes and mesenteric lymph node (MLN) lymphocytes of OVA23-3 mice were pooled in each experimental group, and $CD4^+$ T cells were purified (> 95%) using a MACS cell separation system (Miltenyi Biotec). OVA 23-3 $CD4^+$ T cells (1×10^5 cells/well) were cultured with mitomycin C-treated BALB/c splenocytes as antigen-presenting cells (4×10^5 cells/well) and different concentrations of OVA or OVA323-339 peptide in 96-well plates using RPMI 1640 medium containing 5% FCS. Culture supernatants were collected for cytokine measurements 48 hours later. Cytokine concentrations (IL-2, IL-4 and IFN-γ) were evaluated by ELISA as described previously [8].

In our previous study reporting the OVA 23-3 model,

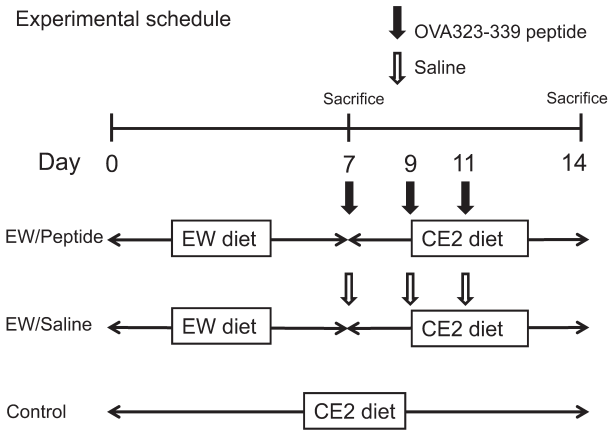

Fig. 1. Experimental schedule for oral administration of OVA323-339 peptide after EW-diet feeding. OVA23-3 mice were orally administered OVA323-339 (EW/Peptide group) or saline (EW/Saline group) three times a week after feeding the EW diet for 7 days. As a control group, OVA23-3 mice were fed their usual mouse diet (CE-2 diet) for 14 days. At days 7 and 14, mice were sacrificed, and spleens and MLNs were harvested for measurement of $CD4^+$ T cell cytokine response.

we showed that splenocytes and MLN lymphocytes from OVA 23-3 mice fed the EW diet for a short period of time produced high levels of IL-4 in response to antigenic stimulation by OVA [5, 9]. Consistent with these results, at day 7, IL-4 production increased in an OVA concentration-dependent manner (data not shown). Splenic $CD4^+$ T cells stimulated with OVA323-339 peptide also produced high levels of IL-4 (Fig. 2). Production of IL-2 decreased, and production of IFN-γ increased slightly. Similar results were observed for MLN $CD4^+$ T cells (data not shown). These results indicated that OVA23-3 mice fed the EW diet for 7 days showed predominant Th2 responses, and suggested that this IL-4 response was due to the $CD4^+$ T cells recognizing the OVA323-339 epitope.

Next, we examined whether oral administration of the OVA323-339 peptide affected the Th2 response elicited by feeding the EW diet. In the spleen, the $CD4^+$ T cell IL-4 cytokine production of the EW/Peptide group at day 14 was significantly lower than that of the EW/Saline group, while no difference was found in IL-2 and IFN-γ production (Fig. 3). On the other hand, the EW/peptide group produced similar amounts of IL-4 to the EW/Saline group in the MLN (data not shown). These data indicate that oral administration of OVA323-339 peptide is capable of inhibiting systemic IL-4 production induced by the EW diet.

In this study, we investigated the efficacy of the T cell epitope OVA323-339 for OIT using the OVA23-3 model. Our data indicated that the OVA323-339 peptide might

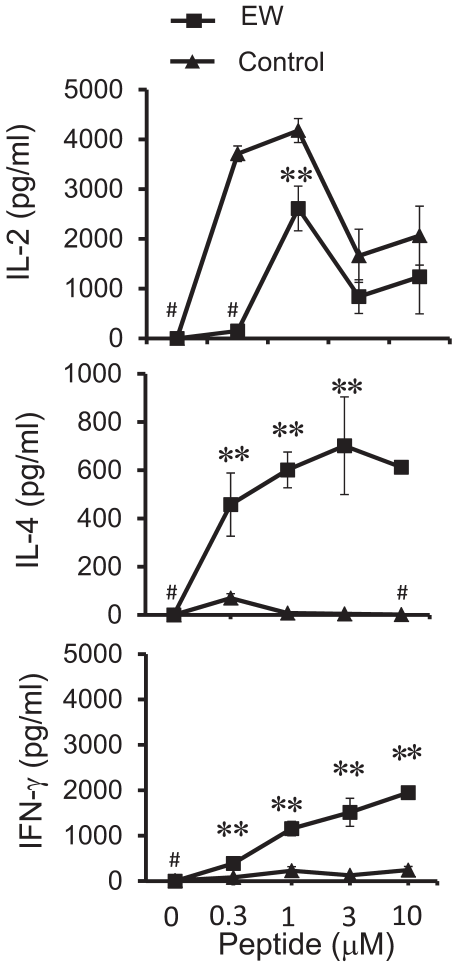

Fig. 2. Cytokine response of OVA23-3 mice fed the EW diet for 7 days. Splenic CD4+ T cells pooled from three mice were incubated with antigen-presenting cells and OVA323-339 peptide. IL-2, IL-4, and IFN-γ in culture supernatants were measured by ELISA. Data are expressed as means ± SD. These data represent 2 independent experiments. To compare the results of the EW group and control group, the Student's t-test was used. **p<0.01. #, Not detected.

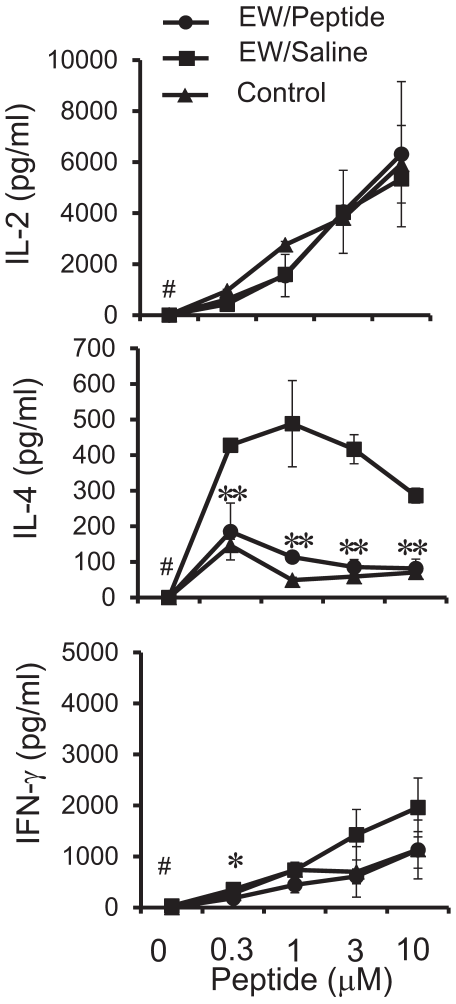

Fig. 3. Effect of oral administration of OVA323-339 peptide on the cytokine response after EW-diet feeding in OVA23-3 mice. Splenic CD4+ T cells pooled from three mice were incubated with antigen-presenting cells and OVA323-339 peptide. IL-2, IL-4 and IFN-γ in culture supernatants were measured by ELISA. Data are expressed as means ± SD. These data represent 2 independent experiments. To compare the results of the EW/Peptide group and EW/Saline group, the Student's t-test was used. *p<0.05. **p<0.01. #, Not detected.

have the ability to suppress systemic Th2 responses and the potential for use as a treatment for allergy to OVA.

We previously reported that OVA23-3 mice fed the EW diet showed enteropathy and IL-4 production in the MLN [5]. Knight et al. demonstrated that transfer of MLN CD4+ T cells from gastrointestinal food allergy model mice to naïve mice induced diarrhea in recipient mice by subsequent oral administration of OVA [10]. In this study, IL-4 production of MLN CD4+ T cells in response to EW-diet feeding was not suppressed in the EW/Peptide group, suggesting that it is difficult to inhibit food-allergic diarrhea by feeding of a T cell epitope peptide of an allergen. Further improvements of the methods such as the doses and frequency of peptide administration may make it possible to suppress the IL-4 production in the MLN and intestinal inflammation.

Infants represent the largest group suffering from food allergy. For infants, it is difficult to administer a peptide

prior to prevent the onset of disease. Hence, we focused on treatments in this study and not prophylaxis. In clinical tests, children with food allergy were fed dietary food containing an allergen to induce oral tolerance. Some patients could successfully establish oral tolerance, while some patients could not acquire tolerance. In unfortunate cases, the patients suffered from side effects [11]. In the present study, Th2 responses were elicited in OVA23-3 mice in response to feeding of the EW diet, and the mice were subsequently administered OVA323-339 orally. As a result, systemic Th2 responses were suppressed by OVA323-339. The results in the present study suggest that the T cell epitope peptide was able to induce a decrease in splenic IL-4 response. OIT using a T cell epitope peptide may be safe because it does not contain intact IgE epitopes. The OVA323-339 region contains cleavage sites of trypsin, chymotrypsin and pepsin, so the OVA323-339 peptide may be degraded to some extent. Nevertheless, systemic Th2 responses were suppressed by oral administration of OVA323-339 peptide, suggesting that the peptide escaped complete digestion and that the OVA peptide (or partly digested OVA peptide(s)) could bind to MHC class II molecules. Our study suggests that the OVA323-339 peptide may have the ability to induce systemic tolerance under the condition of predominant Th2 responses, which may be valuable data for treatment of infantile food allergy.

Although our data demonstrated that IL-4 production was reduced by OVA323-339 in the spleen, we have not elucidated the mechanisms of this suppression. The mechanisms involved in OIT are poorly understood. In our study, IL-4 production was reduced significantly by oral administration of OVA323-339, but that of IFN-γ did not differ, which was similar to a recent study using ovomucoid peptides [4]. Since oral tolerance induced by feeding of an antigen before sensitization generally results in inhibition of Th1 responses [12], this phenomenon may be a feature of "oral tolerance" induced by our T cell epitope peptide after sensitization. In a future work, we will have to make clear the mechanism of IL-4 suppression. It would be interesting to examine if immunoregulatory mechanisms differ in the case of oral administration of a peptide or intact protein, before or after sensitization.

In summary, we showed that oral administration of OVA323-339 peptide has the ability to inhibit systemic IL-4 response after the elicitation of predominant Th2 responses. Our study may be valuable data for OIT of food allergy.

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REFERENCES

1. Nowak-Węgrzyn A, Sampson HA. 2011. Future therapies for food allergies. J Allergy Clin Immunol 127: 558–573. [Medline] [CrossRef]
2. Takagi H, Hiroi T, Yang L, Tada Y, Yuki Y, Takamura K, Ishimitsu R, Kawauchi H, Kiyono H, Takaiwa F. 2005. A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. Proc Natl Acad Sci USA 102: 17525–17530. [Medline] [CrossRef]
3. Suzuki K, Kaminuma O, Yang L, Takai T, Mori A, Umezu-Goto M, Ohtomo T, Ohmachi Y, Noda Y, Hirose S, Okumura K, Ogawa H, Takada K, Hirasawa M, Hiroi T, Takaiwa F. 2011. Prevention of allergic asthma by vaccination with transgenic rice seed expressing mite allergen: induction of allergen-specific oral tolerance without bystander suppression. Plant Biotechnol J 9: 982–990. [Medline] [CrossRef]
4. Rupa P, Mine Y. 2012. Oral immunotherapy with immunodominant T-cell epitope peptides alleviates allergic reactions in a Balb/c mouse model of egg allergy. Allergy 67: 74–82. [Medline] [CrossRef]
5. Nakajima-Adachi H, Ebihara A, Kikuchi A, Ishida T, Sasaki K, Hirano K, Watanabe H, Asai K, Takahashi Y, Kanamori Y, Shimojo N, Matsuda H, Kohno Y, Hachimura S, Kaminogawa S. 2006. Food antigen causes TH2-dependent enteropathy followed by tissue repair in T-cell receptor transgenic mice. J Allergy Clin Immunol 117: 1125–1132. [Medline] [CrossRef]
6. Yang M, Mine Y. 2009. Novel T-cell epitopes of ovalbumin in BALB/c mouse: potential for peptide-immunotherapy. Biochem Biophys Res Commun 378: 203–208. [Medline] [CrossRef]
7. Shimojo N, Katsuki T, Coligan JE, Nishimura Y, Sasazuki T, Tsunoo H, Sakamaki T, Kohno Y, Niimi H. 1994. Identification of the disease-related T cell epitope of ovalbumin and epitope-targeted T cell inactivation in egg allergy. Int Arch Allergy Immunol 105: 155–161. [Medline] [CrossRef]
8. Sato A, Hashiguchi M, Toda E, Hachimura S, Kaminogawa S. 2003. CD11b + Peyer's patch dendritic cells secrete IL-6 and induce IgA secretion from naive B cells. J Immunol 171: 3684–3690. [Medline]
9. Shida K, Hachimura S, Ametani A, Ishimori M, Ling M, Hashiguchi M, Ueda Y, Sato T, Kumagai Y, Takamizawa K, Habu S, Kaminogawa S. 2000. Serum

IgE response to orally ingested antigen: a novel IgE response model with allergen-specific T-cell receptor transgenic mice. J Allergy Clin Immunol 105: 788–795. [Medline] [CrossRef]

10. Knight AK, Blázquez AB, Zhang S, Mayer L, Sampson H, Berin MC. 2007. CD4 T cells activated in the mesenteric lymph node mediate gastrointestinal food allergy in mice. Am J Physiol Gastrointest Liver Physiol 293: G1234–G1243. [Medline] [CrossRef]
11. Patriarca G, Nucera E, Roncallo C, Pollastrini E, Bartolozzi F, De Pasquale T, Buonomo A, Gasbarrini G, Di Campli C, Schiavino D. 2003. Oral desensitizing treatment in food allergy: clinical and immunological results. Aliment Pharmacol Ther 17: 459–465. [Medline] [CrossRef]
12. Faria AM, Weiner HL. 2005. Oral tolerance. Immunol Rev 206: 232–259. [Medline] [CrossRef]