

Dietary unripe apple polyphenol inhibits the development of food allergies in murine models

Hiroshi Akiyama^{a,*}, Yuji Sato^{a,1}, Takahiro Watanabe^a, Megumi H. Nagaoka^a, Yasuo Yoshioka^a, Toshihiko Shoji^b, Tomomasa Kanda^b, Kiyoshi Yamada^c, Mamoru Totsuka^c, Reiko Teshima^a, Jun-ichi Sawada^a, Yukihiro Goda^a, Tamio Maitani^a

^a National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku Tokyo 158-8501, Japan

^b Fundamental Research Laboratory, Asahi Breweries, LTD., 1-21 Midori 1-cho, Moriya, Ibaraki 302-0106, Japan

^c Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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Abstract The incidence of type I allergic disorders has been increasing worldwide, particularly, the hypersensitivity to food. We first showed that apple condensed tannin (ACT) intake would inhibit the development of the oral sensitization and that the inhibition could correlate with the rise in the population of TCR $\gamma\delta$ -T cells in the intestinal intraepithelial lymphocytes (IEL) using W/W^V mice and B10A mice which were ovalbumin (OVA)-orally sensitized. Serum OVA-specific immunoglobulin E and immunoglobulin G1 titers in the OVA-orally sensitized W/W^V and B10A mice ad libitum fed ACT were extremely inhibited compared to those of the control. The ACT intakes of OVA-sensitized W/W^V and B10A mice inhibited the immediate reduction of the body temperature or the rise in serum histamine induced by active systemic anaphylaxis. The proportions of the TCR $\gamma\delta$ -T cells in the IEL of the OVA-orally sensitized W/W^V and B10A mice ad libitum fed ACT were significantly greater than that in the controls. Furthermore, ACT feeding by itself could induce the rise in the percentage of the TCR $\gamma\delta$ -T cells among the IEL of the W/W^V and B10A mice. This suggests that the ACT intake may prevent the development of food allergies and this effect could be correlated with the rise in the percentage of TCR $\gamma\delta$ -T cells among the IEL.

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Keywords: Apple condensed tannin; IgE; Food allergy; Oral sensitization; Intestinal intraepithelial lymphocytes; TCR $\gamma\delta$ -T cell

1. Introduction

The apple contains several phenolic substances, i.e., chlorogenic acid, catechin, epicatechin, phlorizin, rutin, flavonoids, and condensed tannins [1–7]. Osada et al. demonstrated that

apple condensed tannins (ACT) are contained in unripe apples at a ten times higher level than in the ripe ones [8]. ACT is a mixture of oligomers consisting of chains of flavan-3-ol units mainly linked through C4–C8 (or C6) bonds [9]. Some of these substances have physiological and pharmacological activities. Several authors have reported that oligomeric catechins, such as ACT, showed antioxidant activities [10,11]. We have also shown that ACT had an inhibitory effect on histamine release from both rat basophilic leukemia (RBL-2H3) cells by antigen stimulation and rat peritoneal mast cells stimulated by compound 48/80 [12,13] and that ACT intake would improve the symptoms of atopic dermatitis (AD) in patients with AD [14]. These findings collectively indicate that ACT intake may have anti-allergic effects with uniquely active characteristics.

We have previously shown that mast-cell-deficient WBB6F1-W/W^V mice (W/W^V mice) and B10A mice can be efficiently sensitized by the oral administration of an antigen in the form of gavage [15,16]. These mice should be good models for studying the effect of food and drug substances on the development of oral sensitization by food allergens. In this study, we examined the effects of the ACT feeding on the oral sensitization by food allergens and on the intestinal intraepithelial lymphocytes (IEL) using flow cytometry (FCM) analysis.

2. Materials and methods

2.1. Mice

Female WBB6F1-W/W^V mice (6 weeks, W/W^V mice) and female B10A mice (6 weeks) were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan) and kept under pathogen-free conditions in our animal facility for 1 week before use. The mice were maintained in a temperature (23–25 °C), humidity (40–60%) and light-controlled environment with free access to a Mouse Flat (MF) diet (SLC) and water. The ACT was ad libitum administered to the W/W^V mice in the form of the solution dissolved in distilled water at levels of 0%, 0.1% ACT, 0.5% ACT and 1.0% ACT in terms of the dose-dependent study. The control group ($n = 5$, 0%), and three ACT groups (each $n = 5$, 0.1%, 0.5% and 1.0%) had free access to water and each ACT solution with the MF diet. These mice were sensitized by the administration of 1.0 mg ovalbumin (OVA; grade V, Sigma Chemical Co, St. Louis, MO) by daily gavage for 9 weeks. To assess the effect of only the ACT intake on the population of the lymphocytes in the IEL in W/W^V mice, the 1.0% ACT feeding was investigated without oral sensitization to OVA for 2 weeks or 9 weeks in W/W^V mice.

*Corresponding author. Fax: +81 03 3707 6950.

E-mail address: akiyama@nihs.go.jp (H. Akiyama).

¹ Hiroshi Akiyama and Yuji Sato contributed equally to this work.

Abbreviations: ACT, apple condensed tannins; IEL, intestinal intraepithelial lymphocytes; OVA, ovalbumin; IgE, immunoglobulin E; IgG1, immunoglobulin G1; IgG2a, immunoglobulin G2a; FCM, flow cytometry; ASA, active systemic anaphylaxis; MF, Mouse Flat

For a similar examination using B10A mice, the ACT was ad libitum administered to the mice with levels of 1.0% ACT. The control group ($n = 6$) and ACT group ($n = 6$) had free access to water and each ACT solution with the MF diet. The B10A mice were also sensitized by the administration of 1.0 mg OVA by daily gavage for 9 weeks. To assess the effect of only the ACT intake on the population of the lymphocytes in the IEL of the B10A mice, the 1.0% ACT feeding was investigated without oral sensitization to OVA for 9 weeks in the B10A mice.

For the examination of the W/W^V and the B10A mice, the blank group (each $n = 5$ –6) normally had free access to water and the MF diet without the oral sensitization of OVA for 9 weeks. The care and use of the experimental animals in this study followed “The Ethical Guidelines of Animal Care, Handling and Termination” prepared by the National Institute of Health Sciences (NIHS) in Japan.

2.2. Sample preparations

ACT from unripe apples was obtained according to the method reported by Ohnishi-Kameyama et al. [9].

2.3. ELISA for mouse serum anti-OVA IgE, IgG1 and IgG2a titer

The mouse serum titers of anti-OVA immunoglobulin E (IgE), immunoglobulin G1 (IgG1), and immunoglobulin G2a (IgG2a) were determined in triplicate in a 96-well microtiter plate by a previously reported method [17,18]. The serum OVA-specific antibody titers were calculated by the reciprocal of the serum dilution with the fluorescence intensity at 50% of the maximum level.

2.4. Induction of active systemic anaphylaxis and measurement of the body temperature changes and serum histamine level

The W/W^V or B10A mice orally immunized with OVA were intraperitoneally challenged with 0.2 ml PBS containing OVA (1 mg/mouse) to induce active systemic anaphylaxis (ASA) [15]. The body temperature changes associated with the ASA were monitored with a rectal thermometer for the mice (Shibaura Electronics Co., Ltd., Japan) without general anesthesia. Ten minutes after OVA challenge, the mice were killed and their blood was collected to obtain serum for the histamine determination. The serum histamine concentrations were measured using the post-column HPLC method described by Kawasaki et al. [19].

2.5. Isolation of mouse IEL

The IEL were isolated as previously described by Nagafuchi et al. [20].

2.6. FCM analysis

A three-color analysis of the IEL subsets was performed by a previously reported method [15].

2.7. Statistical analysis

All values are expressed as means $\pm$ standard error of the mean. Statistical comparisons were performed by the Student's *t* test or Scheffe's method after an analysis of variances (ANOVA). In all cases, the probability (*P*) values below 0.05 were considered significant.

3. Results

3.1. Body weight and the amount of feed intake

We examined whether ACT intake affects the body weight and feed intake of the W/W^V and B10A mice. There was no significant difference in the body weight and the amount of feed intake between the control group and the ACT fed group during the experimental period. No specific symptom was observed in the ACT group during the study.

3.2. Serum OVA-specific IgE, IgG1 and IgG2a antibody titer

To investigate whether the ACT intake could affect the development of the OVA-oral sensitization of the W/W^V mice, serum OVA-specific IgE, IgG1 and IgG2a antibody titers in the OVA orally-sensitized mice were determined using indirect ELISA (Fig. 1). In the control group, we confirmed that the OVA-specific IgE, IgG1 and IgG2a antibody titers were substantially detected and the mice were sensitized by the oral administration of OVA in the form of gavage as previously reported. In contrast, the OVA-specific IgE and IgG1 titers of the 1.0% ACT feeding group (1.0% ACT group) were significantly lower than those of the control group. In addition, the OVA-specific IgE and IgG1 titers of the 0.5% ACT group was even lower than those of the control group though the OVA-specific IgE titers were not significantly different from those of the control group. These results suggested that the decrease in the OVA-specific IgE and IgG1 titers by the ACT intake appears to be dose-dependent from 0% ACT feeding to 1.0% ACT feeding. Furthermore, during the same examination of B10A mice, the OVA-specific IgE titer of 1.0% ACT group also were significantly lower than those of the control group (Fig. 2).

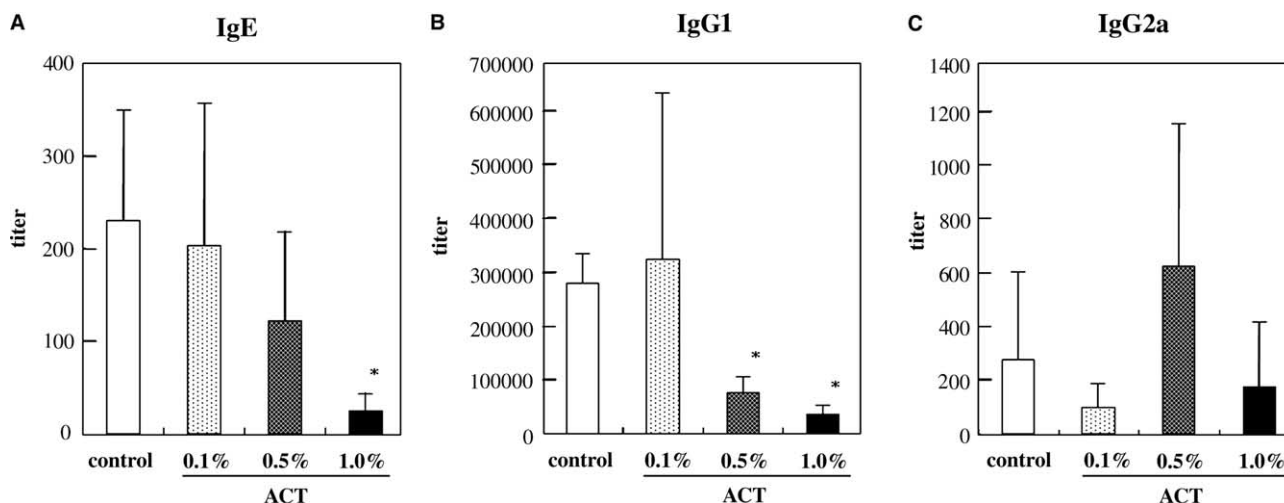


Fig. 1. Effects of ACT intake on serum OVA-specific IgE (A), IgG1 (B) and IgG2a (C) titers in W/W^V mice orally sensitized by OVA. ACT solutions were dissolved in distilled water at levels of 0.1%, 0.5% and 1.0% ACT. Bars represent mean values $\pm$ S.E.M. for five mice. The asterisk indicates significant difference from the control group value (**P* < 0.05).

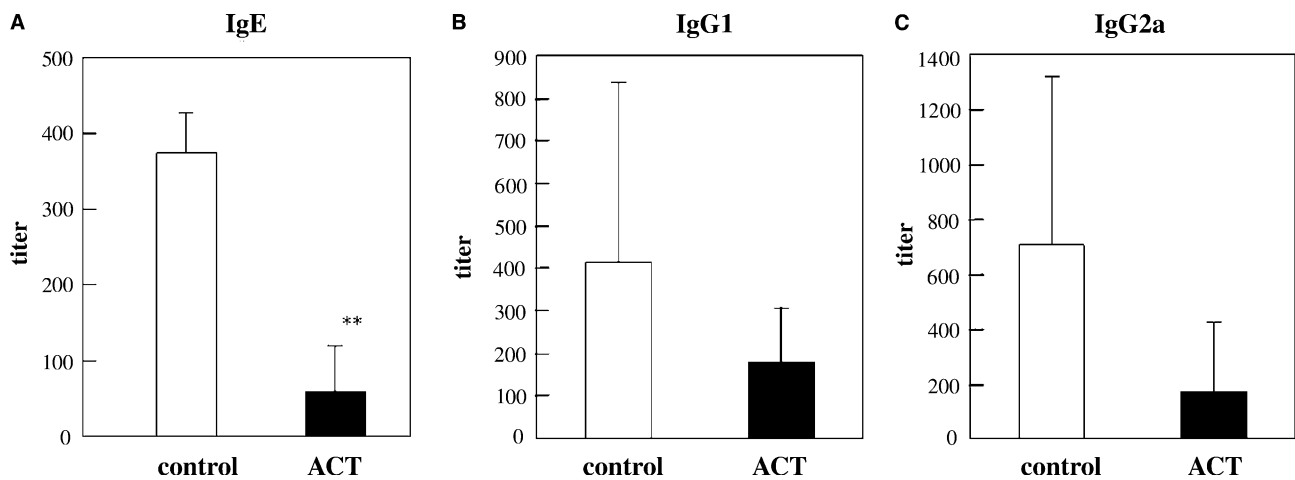


Fig. 2. Effects of ACT intake on serum OVA-specific IgE (A), IgG1 (B) and IgG2a (C) titers in B10A mice orally sensitized by OVA. ACT solutions were dissolved in distilled water at levels of 1.0% ACT. Bars represent mean values $\pm$ S.E.M. for six mice. The asterisk indicates significant difference from the control group value (** $P < 0.01$).

3.3. Body temperature and serum histamine levels after antigen stimulation

To investigate the effects of ACT on the OVA-induced hypersensitivity reaction in this model, the body temperatures of the sensitized W/W^V or B10A mice were measured every minute for 10 min after intraperitoneal challenge with 1 mg of OVA. As shown in Fig. 3, intense hypothermia developed in the control group after challenge for both the W/W^V and B10A mice. However, there was no hyperthermia in the 1.0% ACT group for both W/W^V and B10A mice. Furthermore, a marked increase in serum histamine occurred in B10A mice undergoing antigen challenge. The serum histamine level in the 1.0% ACT group was significantly lower than that in the control group after antigen stimulation of the B10A mice (Fig. 4).

These results suggest that 1.0% ACT intake could prevent the development of the food allergies in these models and could be involved in the inhibition of ACT feeding on the oral sensitization.

3.4. Flow cytometric analysis of the IEL in W/W^V and B10A mice

To examine the mechanisms involved in the inhibition of oral sensitization by the ACT intake, IEL were isolated from the small intestine of the normal W/W^V and B10A mice and

their lymphocytes were analyzed by FCM. As previously reported [15], the proportion of the TCR $\gamma\delta$ (TCR $\alpha\beta$ [−] $\gamma\delta$ ⁺)T cells in the IEL of the W/W^V mice without oral sensitization was approximately 3.0–5.0% of the blank group at the same experimental point (blank in Table 1). As shown in Table 1, the

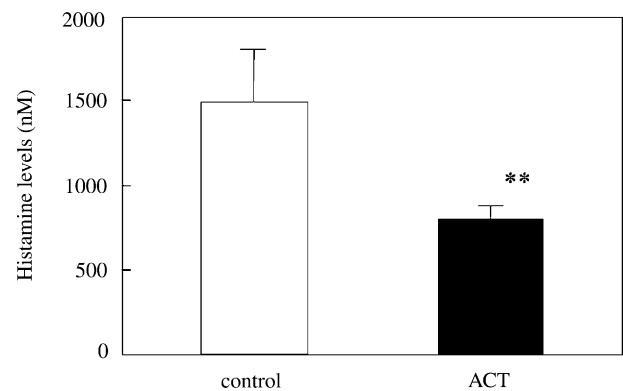


Fig. 4. Determination of serum histamine level of B10A mice after active systemic anaphylaxis. Asterisk indicates significant difference from control value (** $P < 0.01$). Bars represent mean values $\pm$ S.E.M. of four mice.

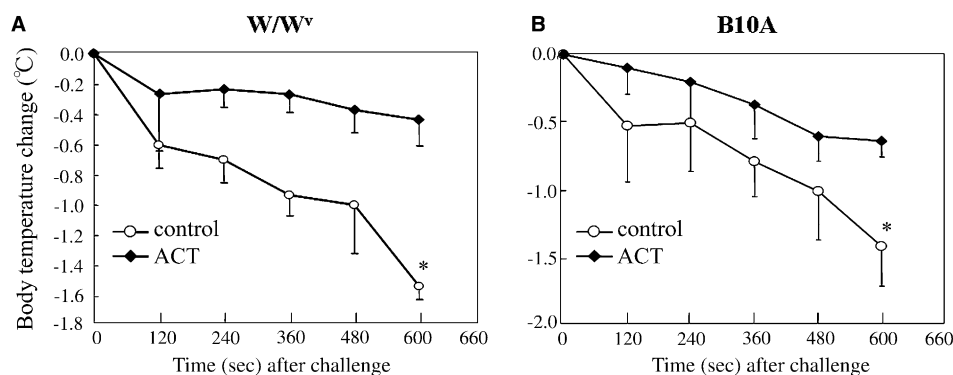


Fig. 3. Changes in body temperature on the sensitized W/W^V mice (A) and B10A mice (B) after the antigen stimulation. *Significantly different from control at $P < 0.05$. Each value represents mean values $\pm$ S.E.M. of five mice.

Table 1
Flow cytometric analysis of the IEL in W/W^V mice

	Blank	Control	0.1% ACT	0.5% ACT	1.0% ACT	1.0% ACT (non-oral sens. for 9 weeks)	1% ACT (non-oral sens. for 2 weeks)
CD3 ⁺ β7 ⁺ (T cell)	86.6 ± 6.0	82.6 ± 5.1	84.5 ± 5.5	88.9 ± 3.6	90.1 ± 2.2*	90.7 ± 2.5*	89.4 ± 3.8*
CD4 ⁺ CD8 ⁺ (TCRαβCD4)	13.1 ± 2.9	15.8 ± 5.9	12.9 ± 5.9	16.4 ± 4.4	10.7 ± 2.0	7.9 ± 3.0*	8.0 ± 2.4*
CD4 ⁺ CD8 ⁺ (TCRαβCD4CD8αα)	24.1 ± 9.2	29.1 ± 5.7	29.0 ± 8.1	33.8 ± 5.1	25.3 ± 7.5	24.7 ± 12.4	10.3 ± 4.2
CD8α ⁺ CD8β ⁺ (CD8αα)	34.9 ± 7.0	37.2 ± 6.1	38.3 ± 4.7	44.6 ± 5.4	49.9 ± 6.1**	50.7 ± 6.0**	41.7 ± 2.5**
CD8α ⁺ CD8β ⁺ (TCRαβCD8αβ)	39.2 ± 9.8	32.5 ± 8.4	33.9 ± 10.9	30.5 ± 7.1	31.7 ± 8.4	36.5 ± 8.6	43.2 ± 5.1
TCRαβ ⁺ γδ ⁺ (TCRγδ)	3.4 ± 1.2	2.0 ± 0.7	2.2 ± 1.2	3.6 ± 1.0*	10.1 ± 1.7**	9.5 ± 3.9**	11.5 ± 2.9**
TCRαβ ⁺ γδ ⁺ (TCRαβ)	89.4 ± 4.1	90.30 ± 1.45	90.9 ± 1.6	90.7 ± 2.3	84.7 ± 1.9*	85.0 ± 4.8*	80.2 ± 4.6*

ACT was ad libitum administered to the W/W^V mice in the form of a solution dissolved in distilled water at levels of 0%, 0.1% ACT, 0.5% ACT and 1.0% ACT in terms of the dose-dependent study. The 1.0% ACT with non-oral sens. for 9 weeks (*n* = 5) and 1.0% ACT with non-oral sens. for 2 weeks (*n* = 5) were investigated without the oral sensitization of OVA for 9 weeks and 2 weeks, respectively. The values represent mean ± S.E.M. (% (gated)), *, **Significant difference from control at *P* < 0.05 and *P* < 0.01, respectively.

Table 2
Flow cytometric analysis of IEL in B10A mice

	Blank	Control	1.0% ACT	1.0% ACT (non-oral sens. for 9 weeks)
CD3 ⁺ β7 ⁺ (T cell)	88.9 ± 2.0	84.6 ± 7.6	85.4 ± 3.7	85.7 ± 2.3
CD4 ⁺ CD8 ⁺ (TCRαβCD4)	7.1 ± 2.9	8.5 ± 5.2	6.0 ± 3.1	4.0 ± 1.6
CD4 ⁺ CD8 ⁺ (TCRαβCD4CD8αα)	15.9 ± 7.4	15.4 ± 7.7	8.5 ± 4.4	9.1 ± 4.1
CD8α ⁺ CD8β ⁺ (CD8αα)	58.0 ± 4.8	54.6 ± 6.0	59.8 ± 6.1	66.7 ± 2.7**
CD8α ⁺ CD8β ⁺ (TCRαβCD8αβ)	23.0 ± 6.3	22.8 ± 2.7	18.0 ± 2.1**	17.8 ± 2.8*
TCRαβ ⁺ γδ ⁺ (TCRγδ)	27.0 ± 7.1	25.9 ± 5.6	34.6 ± 6.8**	34.5 ± 4.9**
TCRαβ ⁺ γδ ⁺ (TCRαβ)	61.9 ± 8.1	60.6 ± 5.0	52.0 ± 6.7**	53.3 ± 6.1**

The control group (*n* = 6) and ACT groups (1.0% ACT, *n* = 6) had free access to water and 1.0% ACT solution, respectively. The 1.0% ACT with non-oral sens. for 9 weeks (*n* = 6) were investigated without the oral sensitization of OVA. The values represent mean ± S.E.M. (% (gated)), *, **Significantly different from control at *P* < 0.05 and *P* < 0.01, respectively.

decrease in the proportion of TCRγδ-T cells (2.0%) by oral sensitization shown as the control group (Control in Table 1) was also confirmed as previously reported [15]. On the other hand, the proportions of TCRγδ-T cells (10.1%) and CD8αα⁺T cells (49.9%) of the 1.0% ACT group (1.0% ACT in Table 1) were significantly higher than those of the control group. The proportion of TCRγδ-T cells in the 0.5% ACT group was slightly increased compared to that of the control group. These results indicated that the increase in the proportion of the TCRγδ-T cells could appear to be ACT dose-dependent up to the 1.0% ACT feeding. Also, in the B10A mice, we found that the proportions of TCRγδ-T cells (34.6%) of the 1.0% ACT group were significantly higher than those (25.9%) of the control group (Table 2, Fig. 5).

Furthermore, to examine the effect of ACT on the population of IEL, only 1.0% ACT was ad libitum administered to the W/W^V or B10A mice without the oral sensitization with OVA. Surprisingly, the proportions of TCRγδ-T cells (9.5%) and CD8αα⁺T cells (50.7%) of the 1.0% ACT group without oral sensitization were significantly higher than those of the blank group (3.4% for TCRγδ-T cells and 34.9% for CD8αα⁺T cells) that were not being orally sensitized, as shown in Table 1. Also, in the B10A mice, the proportions of TCRγδ-T cells (34.5%) and CD8αα⁺T cells (66.7%) of the 1.0% ACT group without oral sensitization were significantly higher than those of the blank group (27.0% for TCRγδ-T cells and 58.0% for CD8αα⁺T cells). These results suggest that only a 1.0% ACT intake induced an increase in the percentage of

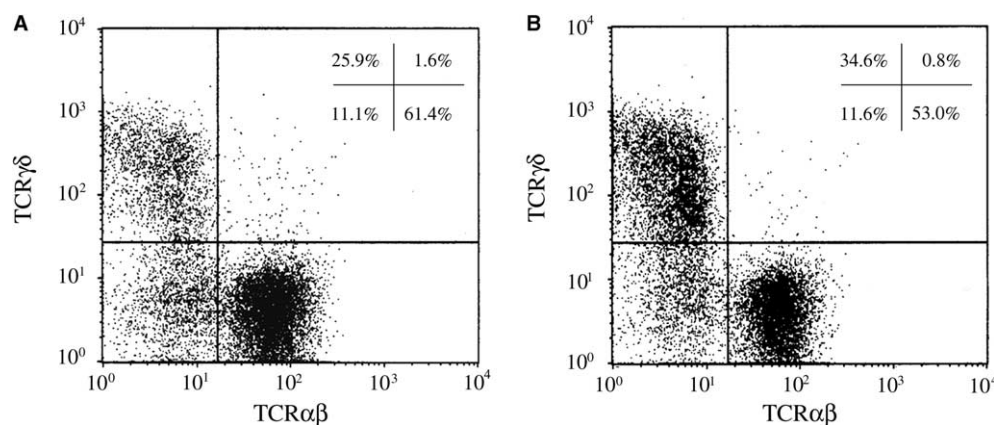


Fig. 5. Expression of TCRαβ and TCRγδ on IEL from B10A mice. IEL were isolated from control group (A) and 1.0% ACT group (B) orally sensitized with OVA. The histograms are representatives of six independent experiments.

TCR $\gamma\delta$ -T cells in the IEL of the W/W^V and B10A mice. Upon the further examination of feeding only 1.0% ACT in the W/W^V mice for two weeks, the proportions of TCR $\gamma\delta$ -T cells (11.5%) and CD8 $\alpha\alpha$ T cells (41.7%) of the 1.0% ACT group with non-oral sensitization were significantly higher than those of the control group for this examination though the proportions of the TCR $\gamma\delta$ -T cells and CD8 $\alpha\alpha$ ⁺T cells in the control group of this examination was 4.2% and 32.3%, respectively (approximately 3–5% for TCR $\gamma\delta$ -T cells and 30–35% for CD8 $\alpha\alpha$ ⁺T cells, respectively, data not shown) and was same as those of the blank group because of not being orally sensitized (Table 1).

3.5. Association of the proanthocyanidin polymerization of the ACT structure for the rise of population of the TCR $\gamma\delta$ -T cells of IEL in the W/W^V mice

In addition, to elucidate the association of the proanthocyanidin polymerization of the ACT structure on the increase in the percentage of the TCR $\gamma\delta$ -T cells of IEL in the W/W^V mice, only a 1.0% monomer proanthocyanidin solution in the mixture of (+)-catechin (14.4%) and (–)-epicatechin (83.6%) extracted from apple was ad libitum administered to the W/W^V mice without the oral sensitization with OVA for two weeks. Interestingly, there was no significant difference between the proportions of the TCR $\gamma\delta$ -T cells (4.8%) and CD8 $\alpha\alpha$ ⁺T cells (33.3%) for only the 1.0% mixture of (+)-catechin and (–)-epicatechin feeding group and those (5.7% and 32.7%, respectively) of the control group.

4. Discussion

In the present study, we first showed that ACT intake would inhibit the development of oral sensitization and that the inhibition could correlate with the rise in the population of TCR $\gamma\delta$ -T cells in the IEL of the W/W^V mice and B10A mice, which are highly susceptible to oral sensitization without using an adjuvant as previously reported [15,16]. In addition, we showed that the ACT intake of OVA-orally sensitized W/W^V and B10A mice inhibited the immediate reduction of the body temperature or the rise of serum histamine levels induced by the antigen stimulation.

Some researchers have developed allergic orally sensitization mouse models using C3H/HeJ mice [21,22]. However, these mice have used cholera toxin as an oral adjuvant in order to be orally-sensitized. Therefore, we considered that the W/W^V and B10A mice should be more appropriate to assess the effect of food substances on the development of oral sensitization and the intestinal mucosal immunity system because these mice can be orally sensitized without using any adjuvants.

Some studies have demonstrated that anaphylactic reactions can be induced in normal and mast-cell-deficient mice, W/W^V mice, after passive transfer of antigen-specific IgG1 antibodies and that these reactions reflect the binding of these antibodies to Fc γ RIII, which can be expressed on the surface of mast cells as well as other cell types such as macrophages, platelets and eosinophils in the mouse [23]. Furthermore, it has been shown that the anaphylactic reactions in mast-cell-deficient mice are involved in the rise of the plasma platelet-activating factor (PAF) [15,24]. In the present study, we are considering that the inhibition of specific IgG1 antibody production by ACT feeding could inhibit the immediate reduction of the body tem-

perature and anaphylactic reactions induced by the reaction among antigen, specific IgG1 antibody and Fc γ RIII expressed on some cells, such as macrophages, platelets and eosinophils in the W/W^V mice. While B10A mice normally have mast cells, the anaphylactic reaction has been thought to be induced by antigens, antigen-specific IgE or IgG1 antibody and Fc ϵ RI expressed on the mast cells because of the immediate rise in the serum histamine levels induced by antigens. Therefore, we conclude that ACT feeding could inhibit specific IgG1 and IgE antibody production induced by oral sensitization and the specific IgG1- or IgE-dependent anaphylaxis on the mast cells in B10A mice.

It has also shown that the percentage of TCR $\gamma\delta$ -T cells in the IEL of the W/W^V mice should be much lower than those of the BALB/c mice, and that the W/W^V mice are also highly susceptible to oral sensitization [15]. Furthermore, we have shown that an additional decrease in the percentage of TCR $\gamma\delta$ -T cells in the IEL cells of the W/W^V mice was also observed in the orally sensitized W/W^V mice [15]. The proportion of TCR $\gamma\delta$ -T cells in the IEL of the W/W^V mice reconstituted with bone marrow cells from C57BL/6J increased much more than that in the untreated W/W^V mice and further extremely inhibited the development of oral sensitization [25]. These findings suggested that the lowering of the population of TCR $\gamma\delta$ -T cells in the IEL of the W/W^V mice could be associated with the high oral sensitivity of the W/W^V mice. In addition, we have shown that ACT intake can induce the proportion of TCR $\gamma\delta$ -T cells and CD8 $\alpha\alpha$ ⁺T cells in the IEL of the W/W^V or B10A mice. Most TCR $\gamma\delta$ -T cells in the IEL are thought to express CD8 $\alpha\alpha$ ⁺ and differentiate into cryptopatches (CPs) [26–28]. Therefore, the present study suggests that the ACT intake mainly increases the population of the TCR $\gamma\delta$ -T cells expressed with CD8 $\alpha\alpha$ ⁺ in the IEL.

Although the function of the TCR $\gamma\delta$ -T cells in the IEL has not been fully elucidated, several reports have suggested the involvement of TCR $\gamma\delta$ -T cells in the IEL in oral tolerance [29–31] and the allergy crisis [32–34]. McMenamin et al. showed that systemic tolerance that is induced by exposure of the respiratory mucosa to OVA activates the TCR $\gamma\delta$ -T cells and CD8⁺ splenic T cells that specifically suppress IgE responses to OVA in adoptive recipient rats and mice [35]. These reports suggest that TCR $\gamma\delta$ -T cells in the IEL would contribute to the induction of oral tolerance.

Considering these findings and our studies, it can be fairly assumed that ACT intake could prevent the development of food allergies through the stimulation of the intestinal mucosal immunity system, and the gut-associated lymphoid tissue correlated with oral tolerance, not due to the physical inhibition of OVA-oral sensitization by ACT.

Condensed tannins are called proanthocyanidins, and have a wide distribution in plants as secondary metabolites. We consume small amounts of these compounds in daily life from fresh fruits such as apple and the processed foods made from these fruits. Rather than nutrients, plant proanthocyanidins are known as the functional food factors that possess a variety of physiological activities such as antioxidant [3–6], anti-allergy, and inhibition against the activities of some physiological enzymes and receptors [36,37]. Most of these parts of the activities of proanthocyanidins appear to depend on their structures and particularly on their degree of polymerization. We showed that the mixture of (+)-catechin and (–)-epicatechin intake in the W/W^V mice for two weeks have no effects

on the percentage of TCR $\gamma\delta$ -T cells in the IEL of the W/W^V mice. This result suggests that the proanthocyanidin polymerization of the ACT structure would be a critical factor for the rise in the percentage of TCR $\gamma\delta$ -T cells of the IEL in the W/W^V mice. The inhibition mechanism of the ACT intake on the oral sensitization in the W/W^V and B10A mice still remains unclear. However, we presumed that the unique structure of the catechin polymerization of ACT should contribute to this activity.

Several clinical medicines prescribed for allergy symptoms, such as corticosteroids, epinephrine, histamine antagonists, and leukotriene synthesis inhibitors, interfere with some steps and lead to the attenuation of the allergic symptoms [6]. However, the effects of these medicines are often short-lived to reduce the allergic responses and induce some side effects. Therefore, a more fundamental means of preventing the development of food allergies should be desirable. Since it has been thought that IgE-mediated food allergies to egg or cow's milk could be developed in early infancy by failing to induce oral tolerance [38], we postulate that the ACT intake during early infancy might prevent the development of food allergies and may continue to inhibit allergic responses to food proteins for a lifetime.

Sampson-HA reported that quantification of food-specific IgE is a useful test for diagnosing symptomatic allergy to egg, milk, peanut, and fish in the pediatric population [38]. Therefore, our present mouse models seem to be useful for examining the effect of bioactive materials to humoral responses of those food allergens.

In conclusion, we showed that ACT intake could inhibit oral sensitization with the rise in the population of TCR $\gamma\delta$ -T cells in the IEL and may fundamentally be used to prevent food allergies. In addition, we believe that ACT can be used as a tool to clarify the role of TCR $\gamma\delta$ -T cells in the IEL. Further studies will be necessary to clarify the mechanism of the induction of oral tolerance by ACT.

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