IgE 抗体解離促進によるイチジク（*Ficus carica* L.）茶のアレルギー抑制効果

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Fig (*Ficus carica* L.) leaf tea suppresses allergy by acceleration disassembly of IgE-receptor complexes

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In this study, I investigated the allergy suppressive effect of tea made from fig (*Ficus carica* L.) leaves. In the rat basophil cell line RBL-2H3, degranulation was significantly suppressed by treatment with fig tea at the same time as addition of IgE antibodies (sensitization). IgE bound to the cell surface was liberated in the medium depending on the treatment time with fig tea. Therefore, it was suggested that the mechanism of action of fig tea is promotion of dissociation of IgE from Fc ε RI receptors. Such a mechanism is novel in food materials. On oral administration to mice, fig tea showed an inhibitory effect on allergic dermatitis. Furthermore, in tests using an atopic dermatitis model in NC/Nga mice, continued administration of fig tea suppressed symptom exacerbation after antigen administration.

Key words: fig (*Ficus carica* L.) tea, type 1 allergy, IgE antibody, atopic dermatitis

An allergy is a physical disorder caused by an inappropriate or excessive immune reaction. The number of patients with allergies increases annually and it is becoming a serious health problem worldwide.[1] The balance between helper T1 (Th1) cells and Th2 cells is considered important for the development of allergic symptoms. These cells differentiate from activated naive T cells (Th0). Whether Th0 cells differentiate into Th1 or Th2 cells is controlled by cytokines and lipid mediators. Interleukin 12 (IL-12) and interferon γ (INF-γ) promote differentiation into Th1 cells. IL-4, IL-13, and prostaglandin E2 (PGE 2) promote differentiation into Th2 cells.[2,3] Th1 cells activate cell-mediated immunity by producing cytokines such as INF-γ and IL-2. Whereas, Th2 cells activate IgE antibody synthesis by producing IL-4, IL-5, and IL-13. In allergic patients, it is presumed that Th2 cells maintain a dominant state and IgE is overproduced.[4–6]

Immediate type (type I) allergy, such as hay fever and bronchial asthma, is an allergy caused mainly by IgE antibodies. Mast cells in mucous membranes and basophil cells in peripheral blood express FcεRI, a transmembrane-type high affinity IgE receptor on the cell surface. When an allergen-specific IgE antibody binds to Fc ε RI on the cell surface, it enters a sensitized state. In sensitized cells, the expression level of FcεRI receptors increases and chemical mediators such as histamine and leukotriene accumulate in cells.[7–10] The allergen invades again, which crosslinks the antibodies bound to the receptors, and the stimulus is transferred into the cell. By crosslinking FcεRI, tyrosine-phosphorylated Lyn phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) and Syk. Phosphorylation of Syk promotes the release of chemical mediators from cells (degranulation) through the activation of MAP kinase and elevation of the intracellular Ca^{2+} concentration.[11–13] The released chemical mediators cause allergic symptoms such as edema, blood pressure lowering, and urticarial.[14]

Type I allergy is suppressed by inhibition of each reaction step, such as IgE antibody production, antibody-receptor binding, intracellular signal transduction, and decreased sensitivity to chemical mediators. Various anti-allergic drugs that apply these mechanisms have been developed. Suplatast- tosylate suppresses the production of IgE antibodies from B cells by suppressing the production of IL-4 and IL-5 in Th2 cells.[15] Omalizumab, an anti-IgE antibody, inhibits binding to FcεRI by capturing free IgE antibodies.[16] Sodium cromoglycate and pemirolast inhibit the activity of phosphodiesterase and phospholipase C, thereby suppressing the elevated Ca^{2+} concentration in mast cells and suppressing degranulation.[17,18] Histamine and leukotriene antagonists

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such as loratadine, oxatomide, and montelukast sodium bind antagonistically to receptors and inhibit allergy. Similarly, functional foods that suppress or alleviate allergies have been developed and applied practically. Methylated catechin contained in 'Benifuki' tea suppresses allergy by inhibiting the phosphorylation of Lyn and Syk and suppressing of expression of FcεRI.[19–21] It has also been reported that ingesting certain lactic acid bacteria (*Lactobacillus plantarum*) improves the Th1/Th2 balance, decreases IgE antibody production, and suppresses allergy.[22]

Fig (*Ficus carica* L.) is a fruit tree of the Moraceae family, and the fruits are eaten raw and processed. On the other hand, leaves have been used for traditional and Chinese medicine for a long time, and various medical properties have been reported.[23] It has been reported that a fig leaf decoction lowers the blood glucose level of patients with type 1 or 2 diabetes mellitus[24–26] and that the solvent extracts of leaves show anti-inflammatory activity in rats.[27] In recent years, it has been revealed that fig leaf extracts suppress the growth of human breast cancer cells MDA-MB-231, and may have wider cancer suppressive effects.[28]

In this study, I analyzed the various physiological activities of fig leaves and, in particular, evaluated the allergy suppressive effect of fig tea processed in the same manner as green tea. Fig tea suppressed type I allergy in cells and *in vivo*. The mechanism of suppression was determined to be promotion of the dissociation of IgE antibody from FcεRI receptor. This is the first report of such a mechanism in food materials.

Materials and Methods

Sample preparation. Fig tea was prepared from the leaves of Fig cultivar 'Masui Dauphine'. The raw material was collected from our orchard (Kawanishi, Hyogo, Japan). After washing with water, the leaves were cut with a ceramic kitchen knife into 2.0-cm squares. The leaves were placed in a metal cage and cooked for 4 min at 90℃ or more in a steam convection oven FCCM 202 (Fujimac, Tokyo, Japan). Thereafter, the leaves were cooled and dried at room temperature for 30 min, kneaded on a hot plate, and dried with a ventilator. The dried leaves were packaged in an aluminum foil laminated pouch and stored at -20℃ until use.

Preparation for fig tea infusion (fig tea), 1.0, 10, and 100 mg of tea leaves were extracted with 1.0 mL of water at 80℃ for 3 min, then filtered through nylon nets (pore size: 48 μm).

Cell culture. The rat basophilic cell line RBL-2H3 were purcahsed from JCRB (Osaka, Japan) and were used for evaluating the degranulation suppressive effect. Cells were cultured in Eagle's minimal essential medium (ThermoFisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Culturing was carried out in 5% CO_2, at 37℃.

Measurement of the release of β-hexosaminidase.

The release of β-hexosaminidase (β-Hex) was measured according to the method of Matsuda *et al*. with some modifications.[29] Before the experiment, RBL-2H3 cells were seeded into 24-well plates (SANPLATEC, Osaka, Japan) at a concentration of 4×10^5 cells/well using a medium containing 0.45 μg/mL mouse monoclonal anti-dinitrophenyl (DNP)-IgE antibody (Merck, Darmstadt, Germany), and were incubated overnight in 5 % CO_2 at 37℃ for sensitization of the cells. Then, cells were washed twice with phosphate-buffered saline (PBS) and incubated in 180 μL Siraganian buffer (119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-1,4-bis-ethanesulfonic acid, 40 mM NaOH, 5.6 mM glucose, 1 mM $CaCl_2$, 0.1% BSA, pH 7.2) for 10 min at 37℃. Then, 20 μL of 0.1 μg/mL DNP-BSA (Merck) as an antigen was added and incubated at 37℃ for 30 min to evoke an allergic reaction (degranulation). The reaction was stopped by cooling on ice for 10 min. Then, 50 μL of supernatant was transferred into a 96-well microplate (ThermoFisher Scientific) and 50 μL of 1.0 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide (Merck) solution (dissolved in 0.1 M citrate buffer, pH 4.5) was added and incubated at 37℃ for 1 h. The reaction was stopped by adding 100 μL of 2.0 M glycine (pH 10.5). The absorbance was measured by a microplate reader (SYNERGY HTX, BioTek Japan, Tokyo, Japan) at 405 nm. The β-Hex release rate of each sample was calculated using the following formula1.

β-Hex release rate (%) = [(T - B) / (E - B)] × 100

Test (T) : DNP-BSA (+), Test sample (+)

Blank (B) : DNP-BSA (−), Test sample (+)

Endogenous (E) : The supernatant of the cell lysate with 1.0% Triton X added to the Blank sample

Each sample was treated immediately after the addition of IgE antibody (sensitization) or 30 min before the addition of DNP-BSA (degranulation). The degranulation suppressive effect of each sample was evaluated as a relative ratio based on the release amount of the control group [DNP-BSA (+), Test sample (−)].

Resensitization test. RBL-2H3 cells were seeded into 24-well plates at a concentration of 4×10^5 cells/well using a medium containing 0.45 μg/mL anti DNP-IgE antibody and fig tea. Cells were incubated overnight in 5

% CO_2 at 37℃. After washing twice with PBS, cells were incubated 3 h using a medium containing antibody only or antibody and fig tea. Then, the β-Hex release amount was measured by the method described above.

Flow cytometric (FCM) analysis. To examine the amount of antibody binding to RBL-2H3 cells, FCM analyses were performed. Cell were seeded into 6-well plates (SANPLATEC) at a concentration of 1.5×10^6 cells/well in a medium containing 0.45 μg/mL anti DNP-IgE (Merck), and were incubated overnight in 5% CO_2 at 37 ℃ for sensitization of the cells. Then, cells were washed twice with PBS, harvested using a cell scraper and suspended in PBS to 1.0×10^6 cells/mL. Next, the cells were labelled with 1.0 μL of 0.5 μg/mL biotin conjugated goat anti-mouse IgE (Beckman Coulter, Pasadena, CA, USA) and 1.0 μL of streptavidin-PE (Beckman Coulter), and analyzed.

Measurement of the amount of dissociated IgE in medium. RBL-2H3 cells were seeded into 6-well plates (SANPLATEC) at a concentration of 1.5×10^6 cells/well in medium containing 0.45 μg/mL anti-DNP-IgE (Merck) and were incubated 1 h in 5% CO_2 at 37℃ for sensitization. After sensitization, excess antibody was removed by removing the medium and washing twice with PBS. Then, medium containing the sample was added, and the medium at each time point was collected. To obtain IgE by immunoprecipitation, 100 ng/mL of biotin conjugated goat anti-mouse IgE (Beckman Coulter) was added to the collected medium and mixed by inversion at room temperature for 1 h. Then, 20 μL of high performance streptavidin sepharose (GE Healthcare Japan, Tokyo, Japan) was added and mixed by inversion for 24 h at 4℃. The sepharose beads were recovered by centrifugation, washed with PBS, and 50 μL of SDS-PAGE sample buffer (180 mM Tris-HCl buffer pH 6.8, 0.2 M SDS, 20% glycerol, 0.01% bromophenol blue) was added, and heated for 5 min at 95℃. The supernatant was collected by centrifugation and an equal volume of Tris-buffered saline (pH 7.4) was added to the SDS-PAGE sample buffer and the protein was developed by running at a constant current of 15 mA for 200 min using a 3%–10% precast gradient gel (ATTO, Osaka, Japan). The IgE antibodies were detected by western blotting using a HRP-conjugated goat anti-mouse IgE antibody (1:10,000; Beckman Coulter). Signal detection and quantification of the IgE were performed using ImageQuant LAS4000 and TL software, respectively (GE Healthcare Japan).

Animal study. Seven-week-old BALB/c female mice were purchased from Charles River Laboratories Japan (Yokohama, Japan) and seven-week-old NC/Nga female mice were purchased from Japan SLC (Shizuoka, Japan). Mice were kept in individual, well-ventilated cages under a specific-pathogen-free environment at a temperature of 23 ± 2℃. The mice were exposed to a 12 h light/dark cycle. The acclimatization period was set to 7 days in all of the experiments. Oral administration of the sample was carried out once a day at an amount of 10 mL/kg. The sample for transdermal administration was prepared by mixing 100 mg of tea powder and 1 g of ointment. The mixture sample was applied once a day at an amount of 20 mg to the right ear. All animal experiments in this study were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and according to the protocol approved by the Animal Experiment Committee of Toyo Institute of Food Technology.

Induction of allergic symptoms and measurement of ear thickness. BALB/c mice were randomly divided into five groups of eight animals as follows: a, oral administration (OA) control (water); b, OA 0.01 g/kg fig tea; c, OA 0.1 g/kg fig tea; d, transdermal administration (TA) control (vehicle); e, TA of 0.1 g/kg mixture sample (Approval No. 2016-A-001). Samples were administered for 10 days after the acclimation period. The day before measurement, mice were injected intradermally with 20 μL of 0.5 μg/mL anti-DNP mouse IgE antibody solution (Merck KGaA) on the ventral side of their right ear. One hour before measurement, 100 μL of 0.5 mg/mL DNP-BSA antigen solution (Bioresearch Technologies, Novart, USA) was injected into the tail vein to evoke allergic symptoms in the right ear.

NC/Nga mice were randomly divided into two groups of eight animals as follows: a, OA control (water); b, OA 0.1 g/kg fig tea (Approval No. 2017-A-002). Samples were administered for 28 days after the acclimation period. NC/Nga mice were injected intradermally with 5 μL of 10 μg/mL mite antigen (Mite Extract-DP: Cosmo Bio, Tokyo, Japan) on the ventral side of their right ear to evoke allergic symptoms on days 0, 3, 7, 10, and 14. During the antigen challenge period (days 0–15), the allergy suppressing effect of fig tea was tested. After the antigen challenge period (days 16–28), the effect of fig tea on recovery from allergic symptoms was evaluated.

In BALB/c mice, the thickness of the ear was measured 1 h before and 1 h after each injection of antigen. In NC/Nga mice, the thickness of the ear was measured every 24 h after challenge up to day 15, then every 2 to 3 days from day 18 onwards. For all ear thickness measurements, an upright gauge (Ozaki, Tokyo, Japan) was used, and an increase in ear thickness was determined by subtracting ear thickness before the first

injection of antigen from that of each time point.

Histological analysis. An ear sample from each BALB/c mouse was obtained on day 10 and fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned at 5 μm, and then stained with hematoxylin and eosin (HE) or toluidine blue (TB).

Statistical analysis. Data are presented as the means ± Standard deviation (SD) for three or more measurements. Statistical analyses were performed using Statcel3 (OMS publishing, Tokyo, Japan) or EXSUS (Version 8.1, CAC Croit, Tokyo, Japan) programs. For comparisons between two groups, the Student's t test was used. For comparisons of differences among multiple groups, one-way analysis of valance followed by Tukey's test or Dunnett's test was used. A p-value less than 0.05 was considered significant.

Results

Fig tea suppressed allergy

Effect of fig tea on cell proliferation

The effect of fig tea on the proliferation of RBL-2H3 cells was investigated. Fig tea at various concentrations (1.0, 10, 100 mg/mL) was added at 1/10 volume of the medium. Concentrations of 1.0 and 10 mg/mL did not inhibit cell proliferation, but 100 mg/mL inhibited cell proliferation from 12 h of culturing. Therefore, a maximum final concentration of 1.0 mg/mL of fig tea was used in subsequent cell culture experiments.

Suppression of allergy by fig tea in cultured cells

The allergy suppressive effect of fig tea was evaluated by measuring the amount of β-Hex released at degranulation of RBL-2H3 cells. β-Hex release was suppressed in a time-dependent manner by treatment with 1.0 mg/mL of fig tea during sensitization. At 24 h after treatment, the release rate became 40% or less, and the strongest inhibitory effect was observed. By contrast, the addition of fig tea at degranulation showed no inhibitory effect (**Figure 1**).

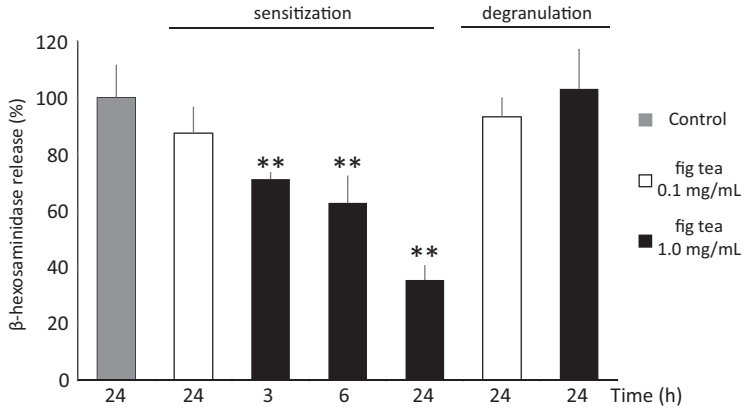

Figure 1. Inhibitory effect of the treatment concentration, time, and timing of fig tea on β-Hex release from antigen-stimulated RBL-2H3 cells.

The results, which represent the mean ± SD (n ≥ 3), were presented as a percentage of the control. Sensitization: addition of sample immediately after the addition of IgE antibody; degranulation: addition of sample 30 min before the addition of antigen (DNP-BSA). Statistically significant results (**$p < 0.01$) were determined by one-way analysis of variance followed by Dunnett's test (multiple comparisons versus a Control).

Suppression of allergy by fig tea in mice

The effect of fig tea on ear swelling was investigated after type I allergy was artificially induced in BALB/c mice. Following OA, the increase in ear thickness of the control group was 98.4 ± 9.9 μm compared with 71.0 ± 13.0 μm in the 0.01 g/kg fig tea dose group and 71.1 ± 6.5 μm in the 0.1 g/kg fig tea dose group. In addition, TA was 92.4 ± 16.4 μm in the fig tea administration group compared with 121.6 ± 8.8 μm in the control group. Using any of the administration methods and concentrations, the swelling of the ear tended to ameliorate by about 75% compared with the control (**Figure 2**). Although there was no significant difference, these results suggested the suppressive effect of fig tea in animals. The fig tea-administered mice showed no difference in body weight or general condition compared with the control mice.

Ears were collected from the OA control group and the OA fig tea group and were histologically analyzed (Figure 3 (a)). Vasodilatation, edema, and neutrophil infiltration were observed in both groups, and there

was no significant amelioration effect on any of these symptoms. Since these symptoms are based on the enhancement of vascular permeability due to the evocation of type I allergy, it was considered that both groups developed type I allergy in this study. However, when the inflammatory symptom score was measured, the fig tea mice showed an improvement compared with the control mice (**Figure 3** (b)). When measuring the number of mast cells, there was no significant difference between the control mice (45.3 ± 6.3 cells/mm^2) and the fig tea-administered mice (46.5 ± 6.2 cells/mm^2).

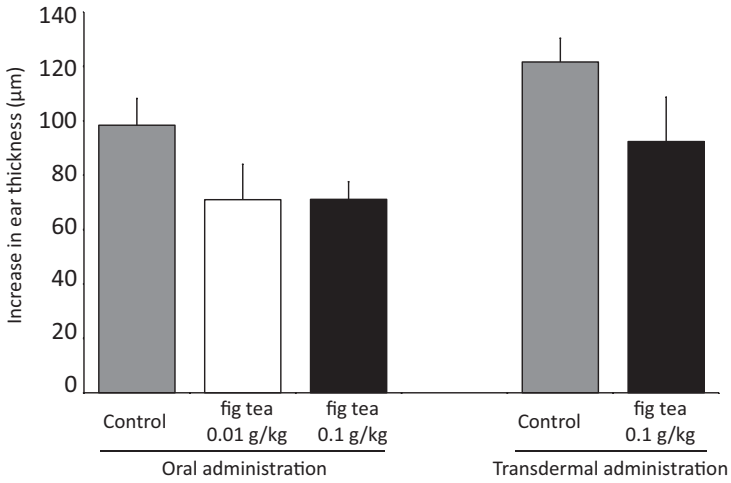

Figure 2. Fig tea efficiently suppresses type I allergy in BALB/c mice.

The allergy suppressive effect of fig tea was evaluated by the measurement of ear thickness in BALB/c mice orally or transdermally administered fig tea for 10 days. Twenty-four hours before measurement, 20 μL of anti-DNP-IgE was injected into the ear for sensitization, and then 100 μL of DNP-BSA was administered through the tail vein to cause allergy. The graph shows the mean ± SD (n = 8). There was no significant difference by Dunnett's test.

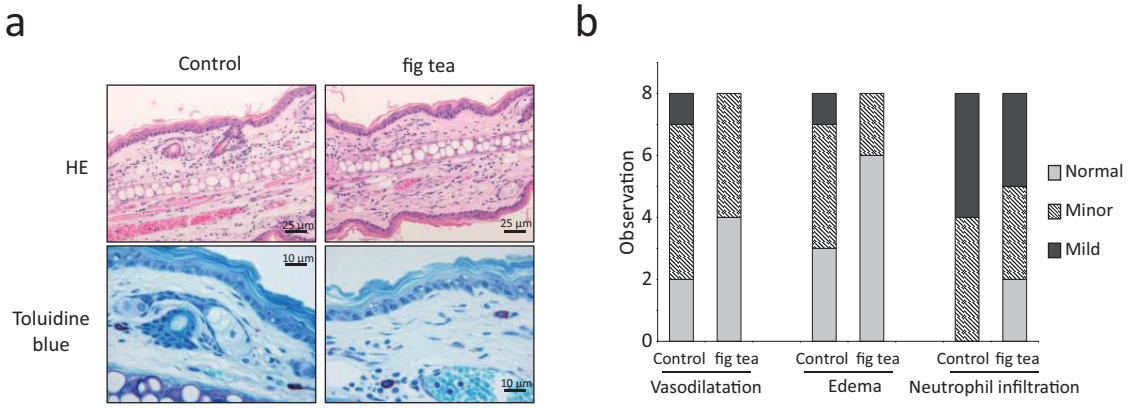

Figure 3. Histological analysis in BALB/c mice.

(a) Representative photomicrographs of ear sections were stained with hematoxylin and eosin (HE) or toluidine blue. The degree of vasodilation, edema, and neutrophil cell infiltration was observed from the HE images, and the number of mast cells per unit area was counted in the toluidine blue-stained images. (b) Symptom score. Each symptom score was determined according to the following criteria. Vasodilatation: slight expansion of the lumen (minor), large expansion of the lumen (mild). Edema: limited in the dermis (minor), presence in the dermis to muscle layer (mild). Inflammatory cell infiltration: a few scattered cells (minor), some aggregated cells (mild).

The mechanism by which fig tea suppresses allergy involves the promotion of IgE dissociation from FcεRI

Effect of fig tea on IgE-FcεRI receptor binding

Since the allergy suppressive effect of fig tea was only observed at sensitization, it was presumed that fig tea acted the binding IgE antibody and FcεRI, which is a receptor of IgE antibody. The amount of IgE antibody bound to the RBL-2H3 cell surface after sensitization was measured by FCM analysis. The amount of antibody binding (mean fluorescence intensity, MFI) was 104.40 ± 36.30 in the control group and 55.65 ± 26.19 in the fig tea-administered group, so the amount of antibody bound in the fig tea group was about half that of the control and this was a statistically significant decrease (p = 0.0467, **Figure 4**). This suggested that the mechanism by which fig tea suppresses allergy may involve a decrease in the

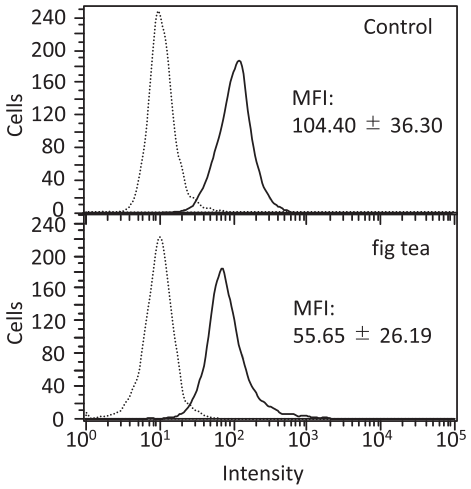

Figure 4. Measurement of the amount of IgE antibody binding by FCM.

To sensitize RBL-2H3 cells, 1 mg/mL of fig tea or water (Control) was added, and after 24 h, the amount of antibody bound to the cell surface was measured. The antibodies were fluorescently labeled with biotin-conjugated goat anti-mouse IgE and streptavidin-PE, and were measured by FCM. The following data was representative of that reported in the article. Solid line: labeled, dashed line: non-labeled. Mean fluorescence intensity (MFI) showed the mean ± SD (n = 4).

binding amount of IgE on the cell surface.

Promotion of the dissociation of IgE from FcεRI receptors

It was speculated that target molecules of active compounds of fig tea were either antibodies or receptors because fig tea reduced the amount of antibody binding on the cell surface. IgE antibodies or cells (receptors) previously exposed to fig tea were used to investigate the effect on suppressing degranulation, but degranulation was not suppressed following either treatment indicating that it was not antibody or receptor alone that was a target molecule (**Figure 5**). Then, it was hypothesized that the target molecule was an IgE-Fc ε RI complex, and I tested whether the addition of fig tea to sensitized cells promoted the dissociation of IgE. After fig tea treatment, the amount of IgE contained in the medium at each elapsed time was detected by western blotting (**Figure 6** (a)). In proportion to the time of exposure to fig tea, the amount of free IgE in the medium increased and reached four times that of the control at 24 h after treatment (**Figure 6** (b)).

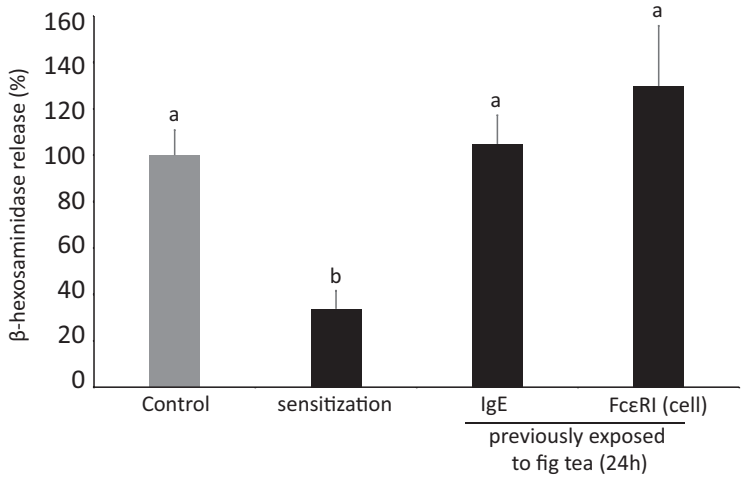

Figure 5. An antibody or receptor alone is not a target molecule for fig tea.

The amount of β-Hex released was evaluated using antibodies or cells previously exposed to 1.0 mg/mL fig tea for 24 h. The results, which represent the mean ± SD (n = 3), were presented as a percentage of the control.
Different letters indicate significant differences ($p < 0.01$). Statistically significant results were determined by one-way analysis of variance followed by Tukey's test.

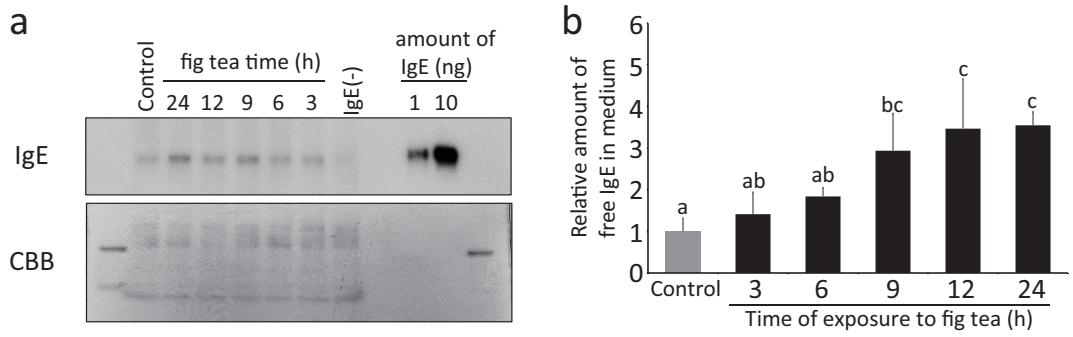

Figure 6. Fig tea accelerates IgE antibody dissociation from Fc ε RI receptor.

(a) Detection of IgE released from Fc ε RI. After antibody binding to RBL-2H3 cells, excess antibody was removed by aspirating the medium and washing. Thereafter, the medium and each sample were added, and the medium at each time point was collected. IgE antibodies were recovered by immunoprecipitation with biotin-conjugated anti-IgE and streptavidin sepharose from the collected medium. IgE was detected by western blotting. After transfer to a nylon membrane, the gel was stained with Coomassie brilliant blue R-250 to detect total protein. (b) The detected IgE was quantified using image analysis software ImageQuant TL. The results, which represent the mean ± SD (n = 3), were presented as a percentage of the control. Different letters indicate significant differences ($p < 0.05$). Statistically significant results were determined by one-way analysis of variance followed by Tukey's test.

Fig tea promoted recovery from allergic symptoms

Sustainability of the antibody dissociation activity of fig tea

A medium containing antibody was added again to RBL-2H3 (resensitization) cells once the antibody had been dissociated by fig tea treatment, and the amount of β-Hex release after 3 h was measured. The β-Hex release rate of the cells to which fig tea was added at resensitization was about 50% that of the control, which was equivalent to the conventional inhibition rate. In addition, the β-Hex release rate was suppressed to 64 % even in cells to which no fig tea was added at resensitization, indicating that there was no difference due to fig tea at resensitization (**Figure 7**).

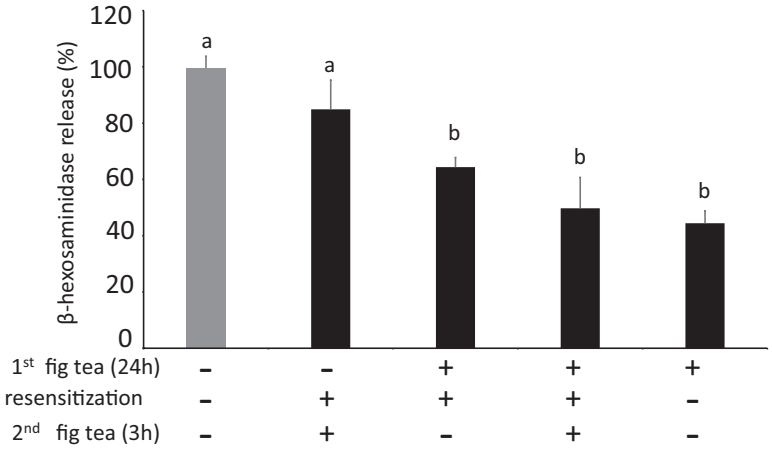

Figure 7. Cells with accelerated antibody dissociation due to fig tea have reduced antibody binding capacity.

The cells from which the antibody was dissociated by 1.0 mg/mL fig tea treatment were resuspended in a medium without fig tea. The antibody only, or antibody and fig tea, were added again (resensitization), and the amount of β-Hex released after 3 h of resensitization was measured.
The results, which represent the mean ± SD (n = 3), were presented as a percentage of the control. Different letters indicate significant differences ($p < 0.05$). Statistically significant results were determined by one-way analysis of variance followed by Tukey's test.

Recovery effect of fig tea in the atopic dermatitis (AD) mouse model

Using NC/Nga mice, an AD model, I investigated the recovery effect of fig tea from AD. The test was divided into the first half (0–14 days) involving the administration of the sample and the antigen, and the second half (15–28 days) involving only administration of the sample. During the first half, ear swelling tended to be suppressed in fig tea-administered mice, and on day 11, ear thickness in the control group was 459.5 ± 22.4 μm compared with 374.6 ± 17.8 μm in the fig tea group, which was significantly suppressed ($p = 0.010$). During the

second half, although the antigen was not administered, ear thickness increased in the control group but not in the fig tea group. At the end of the study (day 28), the thickness of the ear was 683.6 ± 33.2 μm in the control group and 519.6 ± 45.6 μm in the fig tea group, which was significantly suppressed ($p = 0.012$, **Figure 8**).

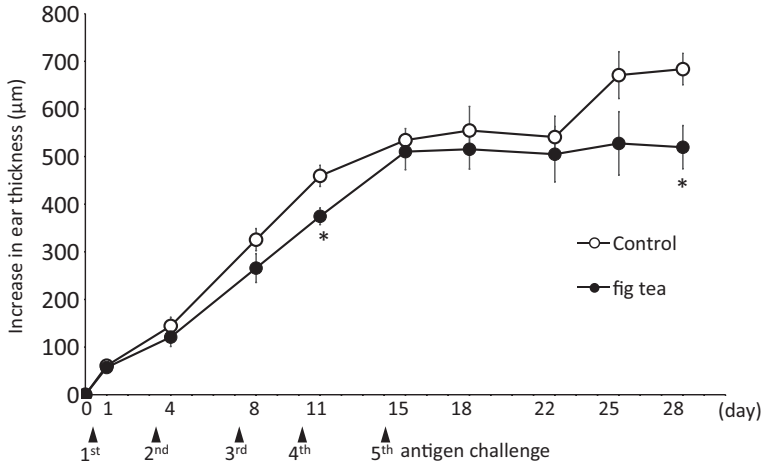

Figure 8. Fig tea alleviates atopic dermatitis (AD) in NC/Nga mice.

10 mg/mL fig tea was orally administered for 28 days to NC/Nga mice that developed AD with mite antigen, and changes in symptoms were investigated. Measurement of ear thickness was performed 24 h after antigen administration until day 15, and every 2–3 days from day 18 onwards. The graph shows the mean ± SD (n = 8). Statistical significance was determined using the Student's t-test to compare samples with the control (*$p < 0.05$).

Discussion

In this study, I investigated the allergy suppressive effect of tea made from fig (*Ficus carica* L.) leaves. In RBL-2H3 cells, degranulation was significantly suppressed by the addition of fig tea upon sensitization. Furthermore, experiments in mice showed the inhibitory effect of fig tea against type I allergy and AD. Previously, the anti-allergic effect of fig tea has only been reported in RBL-2H3 cells, so this study was the first to clarify the effects in living organisms.

Takahashi *et al*. reported that caffeoyl-malic acid, rutin, and isoschaftoside were the major polyphenols in fig leaves.[30] Among these components, it has been reported that rutin has allergy suppressing effects. In AD mice, transdermal administration of rutin suppressed the induction of inflammatory cytokines such as IL-4 and IL-5 that promote allergic reactions, and decreased ear thickness and blood IgE concentration.[31] Extracts of fruits and leaves of *Morinda citrifolia* contained rutin and ursolic acid, and ear edema due to inflammation was reduced in ICR mice following oral administration of these extracts.[32] Taken together, this evidence strongly suggests that rutin is an active component of the anti-allergic effect of fig tea. Furthermore, rutin content of the fig tea was three times higher than that of the leaf extract. Therefore, it was considered that fig tea was more suitable for obtaining an anti-allergic effect.

Fig tea acted only when binding IgE and FcεRI (sensitization), and had no effect on antigen–antibody reactions (degranulation). In addition, FCM analysis revealed that the amount of IgE antibody bound to the cell surface decreased following treatment with fig tea, and western blot analysis revealed that the amount of IgE antibody liberated in the medium increased depending on the treatment time of fig tea. These results suggested that the mechanism by which fig tea suppresses allergy involved promoting the dissociation of IgE antibody bound to the FcεRI receptor and subsequently reducing the association frequency of antigen and antibody. The discovery of food materials with IgE antibody dissociation activity was novel. However, two substances with similar effects have been reported, the anti-allergic agent omalizumab and the designed ankyrin repeat protein (DARP) E2_79, which is an artificially synthesized antibody mimic.[33] Both substances function by binding to IgE antibodies, but their binding sites are different. Although the mechanism of action of these components has not been clarified, it is speculated that a change in the conformation of the IgE-FcεRI complex upon binding to an antibody causes dissociation. In fact, the structural change in the antibody that results from binding of CD23, one of the IgE receptors, to the IgE antibody inhibits binding to FcεRI.[34] Rutin, which is considered to be an active ingredient of fig tea, suppresses the increase in intracellular Ca^{2+} and inhibits the degranulation of RBL-

2H3 cells.[35] Therefore, it is surmised that components other than rutin contribute to the IgE antibody dissociation promoting activity of fig tea.

The degranulation inhibitory effect of fig tea was delayed and became stronger after 12 h of treatment. Whereas, in the antibody re-addition experiment, degranulation was strongly suppressed after 3 h of treatment. Since the IgE antibody dissociation effect of fig tea became stronger after 9 h of treatment, it was suggested that the result of the re-addition experiment reflected the inhibition of binding to the receptor rather than dissociation of the antibody. In other words, fig tea was presumed to have the following two activities: (1) dissociation of antibody from the receptor, and (2) the inhibition of binding, which maintained the structural change in the receptor. Ohnmacht and Voehringer used fluorescent BrdU to show that basophil cell turnover in mice was at least 60 h.[36] If the turnover of basophil cells is not changed by the binding of IgE, the alleviation of allergic symptoms by antibody dissociation and the preventative and ameliorating effects induced by the inhibition of binding to the receptor can be expected in animals that have consumed fig tea. In BALB/c mice, allergic skin inflammation (a type I allergy) developed locally following intradermal administration of IgE to the ear. The mice treated with fig tea showed a tendency to suppress swelling due to inflammation regardless of the method of ingestion. Type I allergy-induced inflammation was mainly caused by mast cell degranulation, suggesting that fig tea acted not only on basophil cells but also on mast cells. In NC/Nga mice, the fig tea administration group showed alleviation of dermatitis and suppression of exacerbation after completion of antigen administration compared with the control group. The skin lesions in NC/Nga mice not only closely resembled AD clinically and histopathologically, but also had similar characteristics such as enhanced sensitivity to IL-4 and phosphorylation of JAK3. Thus, NC/Nga mice are widely used as AD models.[37] The results of this study suggested that fig tea was effective in alleviating symptoms for chronic allergic dermatitis conditions such as AD. According to Tomimori *et al*., in C3H mice and NC/Nga mice that developed inflammation due to repeated administration of 2, 4-dinitrofluorobenzene (DNFB), C3H mice showed rapid improvement of symptoms upon termination of DNFB administration, whereas in NC/Nga mice ear swelling continued until 4 weeks after administration had ended.[38] Since NC/Nga mice were used in this study, it might have taken time for the animals to recover after antigen administration, whereas using C3H mice may give clearer results on the recovery effect of fig tea. Recently, from studies using mice that can selectively remove basophil cells, it was suggested that basophil cells could act as initiator cells rather than effector cells in chronic allergic inflammatory conditions such as AD.[39,40] It is expected that the elucidation of the onset mechanism of chronic allergic inflammation of basophil cells will lead to the elucidation of the *in vivo* mechanism of allergy suppression by fig tea.

Conflicts of interest

There are no conflicts of interest to declare.

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