

Preventive effects of black tea theaflavins against mouse type IV allergy

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Abstract

BACKGROUND: Tea (*Camellia sinensis* L.), one of the most popular beverages, contains various beneficial constituents. We investigated the preventive effects of black tea theaflavins, theaflavin-3-gallate (3-TF) and theaflavin-3,3'-digallate (TFDG), on oxazolone-induced type IV allergy in male ICR mice.

RESULTS: Percutaneous administration of both 3-TF and TFDG at 0.2 mg ear⁻¹ showed significant preventive effects against mouse type IV allergy. Oral administration of these agents at 50 mg kg⁻¹ body weight also showed significant preventive effects against mouse type IV allergy. Oral administration of 3-TF and TFDG at a dose of 50 mg kg⁻¹ body weight prevented the increases in levels of some proinflammatory cytokines, interleukin-12 (IL-12), interferon-gamma (IFN- γ), and tumour necrosis factor-alpha (TNF- α), in the sera and/or ears of mice with type IV allergy. Lowering of serum antioxidant activity in mice with allergic symptoms was also prevented by oral administration of these theaflavins at a dose of 50 mg kg⁻¹ body weight. The anti-allergic mechanisms of action of theaflavins involve inhibition of the fluctuations of cytokines and maintenance of antioxidant status in allergic mice.

CONCLUSION: These results suggest that the theaflavins as well as catechins contribute to the anti-allergic effects of black tea.

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Keywords: black tea; theaflavin; type IV allergy; mouse; cytokine; antioxidant activity

INTRODUCTION

The recent increase in incidence rates of allergic diseases around the world has become an important medical problem. Immune responses can be divided into four types, type I to type IV, based on their mechanisms.¹ Type IV allergy, such as contact hypersensitivity and tuberculosis, includes the most common allergic diseases along with type I allergy, anaphylaxis. Type IV allergy is known as delayed type hypersensitivity and involves cell-mediated immunity controlled by some proinflammatory cytokines released from type 1 T helper (Th1) cells.

Tea (*Camellia sinensis* L.) is one of the most popular beverages around the world. Abe *et al.*² reported that the extracts of green tea and black tea, as well as some catechins present in tea leaves, could prevent picryl chloride-induced mouse ear oedema. Furthermore, extracts of black tea showed preventive effects on sheep red blood cell-induced mouse hind paw oedema. We also reported that the extract of green tea, some catechins, and their *O*-methylated derivatives present in tea leaves could prevent oxazolone-induced mouse ear oedema.^{3,4} These anti-allergic activities of tea catechins were thought to be due to their anti-inflammatory activities and inhibitory effects on the differentiation and proliferation of effector T cells.^{2,5} Black tea extract also showed preventive effects in various animal models of inflammation, such as acute, chronic, exudative and/or proliferative types.⁶ Although catechins are present in both black and green tea, the total amounts of catechins in black tea leaves are low (approximately 30–50% of those in green tea leaves).⁷ During the fermentation process involved in making black tea, catechins are oxidised enzymatically and their dimers, theaflavins, and polymers, thearubigin, are produced. In black tea

leaves, the content of catechins, theaflavins and thearubigin are 40–70 g kg⁻¹, 7–15 g kg⁻¹ and 100–200 g kg⁻¹, respectively.^{7,8} In addition to catechins, these oxidised products also have strong anti-inflammatory effects.^{9,10} Lin *et al.*¹¹ suggested that the galloyl moiety of catechins and theaflavins was essential for their potent anti-inflammatory activities. These reports indicated that not only catechins, but also theaflavins and thearubigin are expected to contribute to the anti-allergic activities of black and green teas.

In this study, we investigated the preventive effects of theaflavin-3-gallate (3-TF) and theaflavin-3,3'-digallate (TFDG), which are theaflavins possessing galloyl moieties in their chemical structures, against mouse type IV allergy induced by oxazolone as well as the effects on the cytokine levels and antioxidant activities in the sera and ears of allergic mice.

MATERIALS AND METHODS

Samples

Theaflavins from black tea leaves, 3-TF and TFDG, were supplied by Unilever Japan KK (Tokyo, Japan). The purities of these compounds

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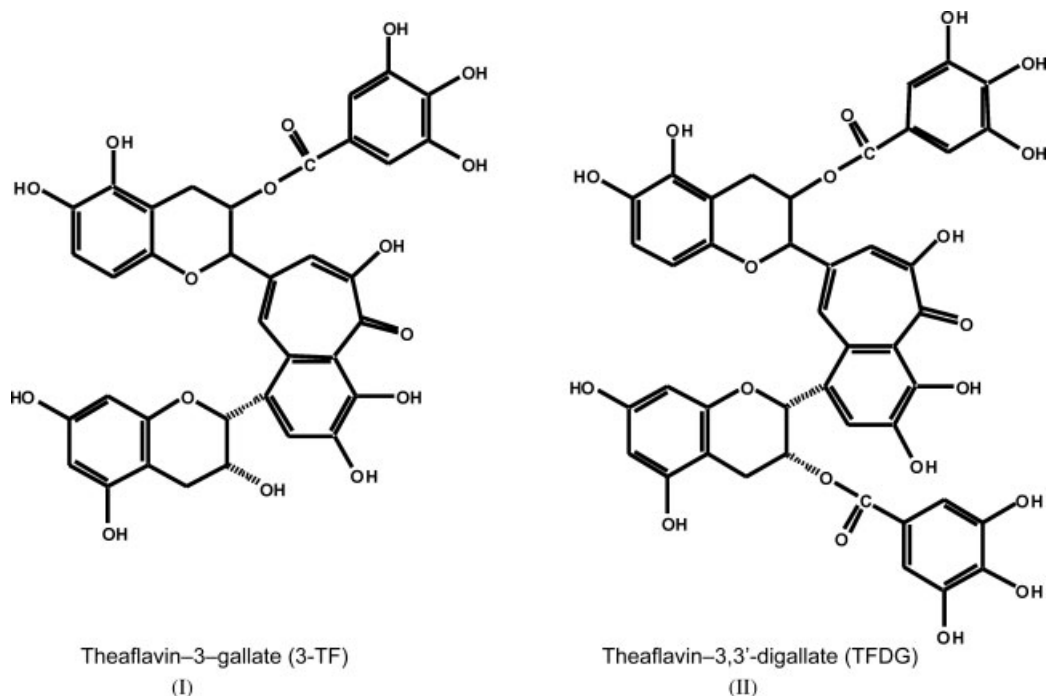


Figure 1. Chemical structures of black tea theaflavins.

were higher than 95%. Chemical structures of 3-TF and TFDG are shown as (I) and (II) in Fig. 1.

Mouse type IV allergic model

Oxazolone-induced oedema of the ear in mice was used as a model of type IV allergy.^{12,13} Oxazolone, a sensitizer, was purchased from Wako Pure Chemical Industries (Osaka, Japan). Male ICR mice, 4 weeks old, were purchased from Japan SLC, Inc. (Shizuoka, Japan). The hair of the abdominal region was carefully shaved off, and 0.1 mL of a 0.5% oxazolone solution in ethanol was applied to the skin (sensitisation). Five days after sensitisation, 20 μ L of a 0.5% oxazolone solution in acetone was applied to both sides of each animal's right ear (challenge). Twenty-four hours after the challenge, the mice were killed under ether anaesthesia, and circular parts (5.0 mm in diameter) were removed from both ears using a punching apparatus. Mouse blood was taken and centrifuged at $1500 \times g$ for 10 min, and the supernatant was used as the serum. The weights of the right and left ears (WR and WL, respectively) were measured and the ear swelling ratio (%) was calculated using the following equation:

$$\text{ear swelling ratio} = \frac{(WR_s - WL_s) / WL_s}{(WR_c - WL_c) / WL_c} \times 100$$

where s refers to the sample and c refers to the control.

In the case of percutaneous administration of the samples to mice, 3-TF and TFDG were dissolved in oxazolone/acetone solution at challenge, and the doses applied were 0.05, 0.1 and 0.2 mg ear⁻¹. Hydrocortisone, a well-known steroid-type anti-inflammatory agent, was also assayed at a dose of 0.1 mg ear⁻¹. A control experiment was performed with only the solvents used for dissolution of the samples. Body weights of mice used in this study were 21.5 ± 1.8 g.

In the case of oral administration of the samples to mice, 3-TF, TFDG, and hydrocortisone were dissolved in 0.5% tragacanth gum solution, and administered orally to mice 1 h prior to the challenge. The doses of theaflavins were 10 and 50 mg kg⁻¹ body weight. Hydrocortisone was also assayed at doses of 50 and 100 g kg⁻¹ body weight. A control experiment was performed with 0.5% tragacanth gum solution. Body weights of mice used in this study were 24.4 ± 2.2 g.

There were five mice in each group. Throughout the experiment, the animals were handled in accordance with *The Guide for the Animal Experiments in Numazu National College of Technology*, which is based on the publication *Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology*.

Determination of cytokines in mouse sera and/or ears

The levels of interleukin-12 (IL-12), interferon-gamma (IFN- γ), and tumour necrosis factor-alpha (TNF- α) in mouse sera and/or ears were determined by enzyme-linked immunosorbent assay (ELISA) with a Mouse IL-12+p40 ELISA Kit (BioSource, San Jose, CA, USA), Mouse IFN γ Instant ELISA (Bender MedSystems, Vienna, Austria), and Mouse TNF- α ELISA Kit (Cosmo Bio Co. Ltd., Tokyo, Japan), respectively.

Determination of antioxidant activities in mouse sera

Male ddY mice, 6 weeks old, were purchased from Japan SLC. The supernatant of 5% mouse brain homogenate in 0.04 mol L⁻¹ phosphate buffer (pH 7.4) was prepared and aliquots of 0.25 mL were mixed with 0.5 mL of phosphate buffer. The mixture with or without 3 μ L of serum from mice used in the allergy experiment, was auto-oxidised at 37 °C for 60 min.¹⁴ The level of lipid peroxide in the reaction mixture was determined by the thiobarbituric acid method¹⁵ and the inhibitory activities in mouse serum against lipid peroxidation were evaluated.

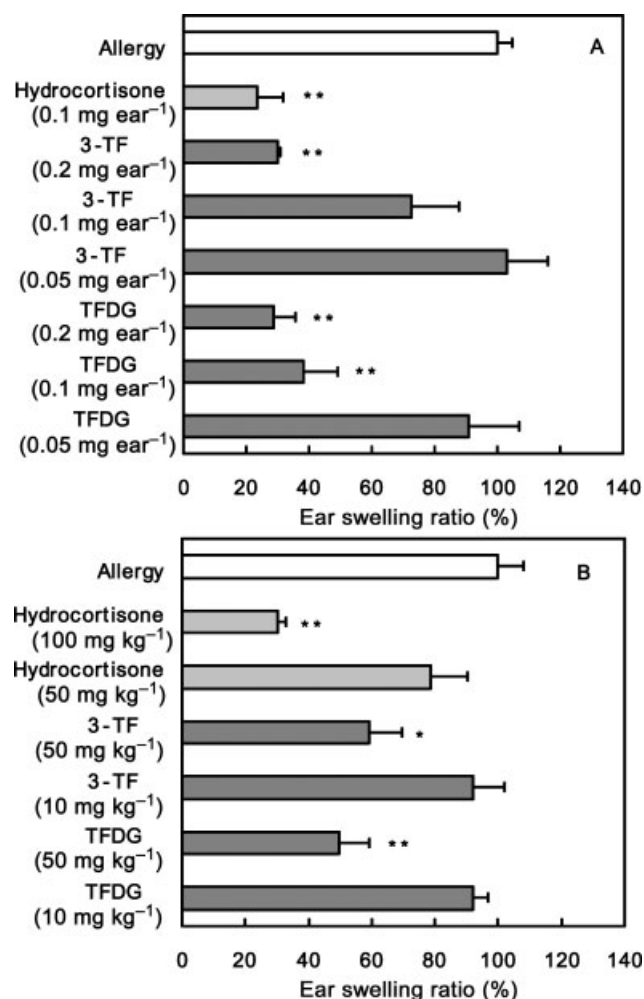


Figure 2. Preventive effects of percutaneous and oral administration of black tea theaflavins on mouse type IV allergy. (A) percutaneous administration; (B) oral administration. Mean $\pm$ SE, $n = 5$. Significant differences from the 'Allergy' group; * $P < 0.05$, ** $P < 0.01$.

Statistical analysis

Differences between the appropriate experimental groups were analysed by the non-parametric Mann-Whitney U test, and $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of percutaneous or oral administration of theaflavins on mouse type IV allergy

The preventive effects of 3-TF and TFDG against type IV allergy in ICR mice after percutaneous administration are shown in Fig. 2A. The administration of TFDG at 0.1 mg ear^{-1} and both 3-TF and TFDG at 0.2 mg ear^{-1} significantly prevented mouse type IV allergy. Their activities were lower than that of hydrocortisone used as a positive control at 0.1 mg ear^{-1} . 3-TF and TFDG at 0.05 , 0.1 and 0.2 mg ear^{-1} showed dose-dependent preventive effects against mouse type IV allergy, and the activities of TFDG tended to be higher than those of 3-TF. The chemical structures of 3-TF and TFDG include one and two galloyl moieties, respectively. Therefore, the galloyl moieties of theaflavins would appear to contribute to the preventive effects of these compounds against type IV allergy. These results were similar to those of our previous study,³ which

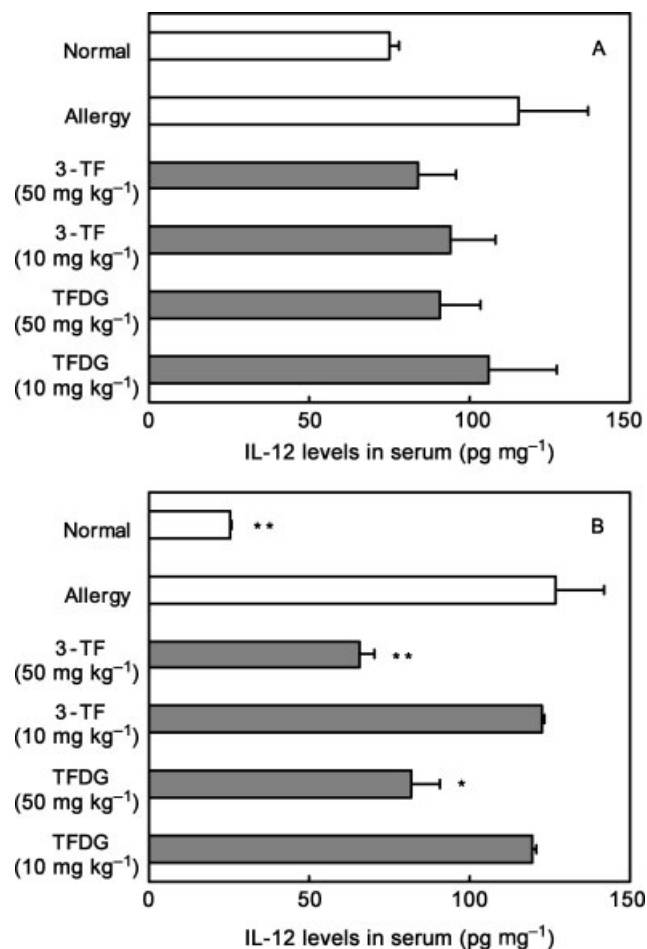


Figure 3. Effects of oral administration of black tea theaflavins on serum and ear IL-12 levels in mice with type IV allergy. (A) Serum; (B) ear. Mean $\pm$ SE, $n = 5$. Significant differences from the 'Allergy' group; * $P < 0.05$, ** $P < 0.01$.

showed that the preventive effects of catechins from tea leaves against mouse type IV allergy were slightly weaker than that of hydrocortisone and the effects of catechins with galloyl moieties were higher than those of catechins without galloyl moieties.

The preventive effects of 3-TF and TFDG on type IV allergy in ICR mice after oral administration are shown in Fig. 2B. The administration of 3-TF and TFDG at 50 mg kg^{-1} body weight significantly prevented mouse type IV allergy. Their activities were higher than that of hydrocortisone at 50 mg kg^{-1} body weight. Hydrocortisone significantly prevented mouse type IV allergy only at 100 mg kg^{-1} body weight. 3-TF and TFDG at 10 and 50 mg kg^{-1} body weight showed dose-dependent preventive effects against mouse type IV allergy. The activity of TFDG tended to be higher than that of 3-TF at 50 mg kg^{-1} body weight. These results were similar with those observed in the experiments with percutaneous administration of theaflavins. Therefore, theaflavins as well as catechins contribute to the antiallergic effects of black tea.

Effects of oral administration of theaflavins on serum and/or ear levels of cytokines in mice with type IV allergy

The effects of oral administration of 3-TF and TFDG at 10 and 50 mg kg^{-1} body weight on the serum and/or ear levels of some proinflammatory cytokines, IL-12, IFN- γ and TNF- α , in mice with type IV allergy induced by oxazolone are shown in Figs 3, 4

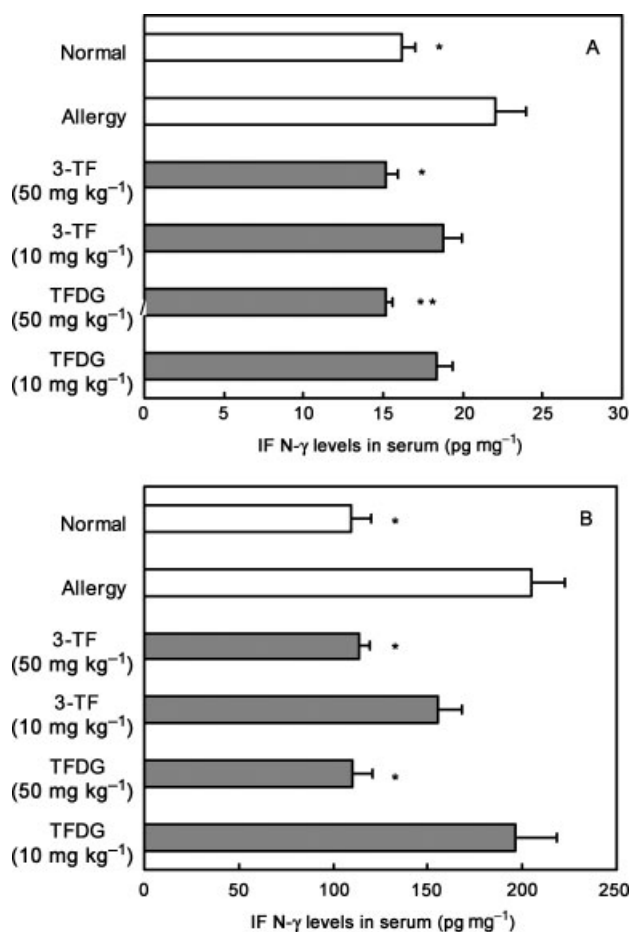


Figure 4. Effects of oral administration of black tea theaflavins on serum and IFN- γ levels in mice with type IV allergy. (A) Serum; (B) ear. Mean $\pm$ SE, $n = 5$. Significant differences from the 'Allergy' group; * $P < 0.05$, ** $P < 0.01$.

and 5, respectively. Severe inflammation is a key process in the development of type IV allergy. Serum IL-12 levels tended to be higher and the ear levels were significantly higher in mice showing intense allergic symptoms than in normal mice (Fig. 3). The oral administration of theaflavins tended to prevent this increase in mouse sera at 10 and 50 mg kg⁻¹ body weight, and showed a significant preventive effect in the ears at 50 mg kg⁻¹ body weight. IL-12 is a cytokine secreted by macrophage-like antigen presenting cells (APCs), and induces the differentiation of naive T cells into Th1 cells. Serum and ear IFN- γ levels were significantly higher in mice showing intense allergic symptoms than in normal controls (Fig. 4). The oral administration of theaflavins tended to prevent this increase in mouse sera and ears at 10 and 50 mg kg⁻¹ body weight and significant effects were observed at 50 mg kg⁻¹ body weight. Ear TNF- α levels tended to be higher in mice showing intense allergic symptoms than in normal controls (Fig. 5). The oral administration of theaflavins significantly prevented this increase in mouse ears at 50 mg kg⁻¹ body weight. At 10 mg kg⁻¹ body weight, ear TNF- α levels of mice administered theaflavins were almost the same or tended to be higher as compared to that of allergic mice, although there was no significant difference between these groups. Serum TNF- α levels in mice could not be detected, because they would be below the detection limit (3 pg mL⁻¹) of the kit used in this study. IFN- γ and TNF- α have multiple actions, such as stimulation of macrophages, Th1 cells and CTL, and suppression of type 2 T helper cells. Oral administration

of 3-TF and TFDG exhibited dose-dependent preventive effects against the increases in levels of these cytokines in mouse sera and ears. The effects of TFDG tended to be higher than those of 3-TF except in the case of IL-12. Therefore, these suppressive effects of theaflavins on the induction of proinflammatory cytokines are thought to contribute to the preventive effects against type IV allergy in mice.

Effects of oral administration of theaflavins on serum antioxidant activities in mice with type IV allergy

Inflammation is thought to be induced by excess active oxygen species, such as the superoxide anion radical and nitric oxide, produced from phagocytes such as macrophages stimulated in an allergic response. It is well known that theaflavins show antioxidative activities^{16,17} as well as catechins.^{18,19} The antioxidant activities in the sera of mice affected by type IV allergy induced by oxazolone are shown in Fig. 6. The antioxidative activities were significantly lower in allergic mice than in normal controls. Oral administration of 3-TF and TFDG dose-dependently prevented the decreases in serum antioxidant activities and significant effects were observed at 50 mg kg⁻¹ body weight. The effects of TFDG tended to be higher than those of 3-TF. The excess production of superoxide anion radicals and nitric oxide by macrophages and neutrophils is thought to provoke inflammation in the neighbouring tissues.^{20,21} The enhancement

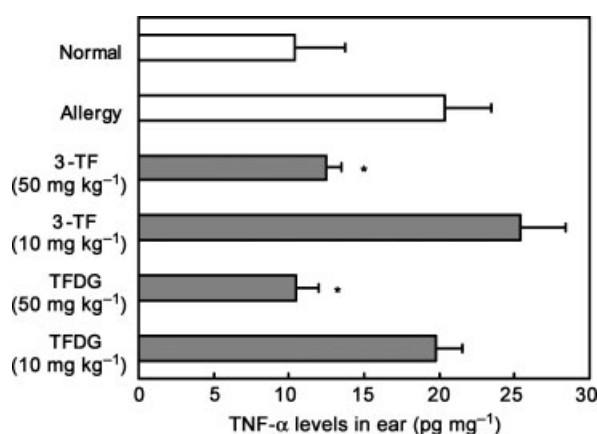


Figure 5. Effects of oral administration of black tea theaflavins on ear TNF- α levels in mice with type IV allergy. Mean $\pm$ SE, $n = 5$. Significant differences from the 'Allergy' group; * $P < 0.05$.

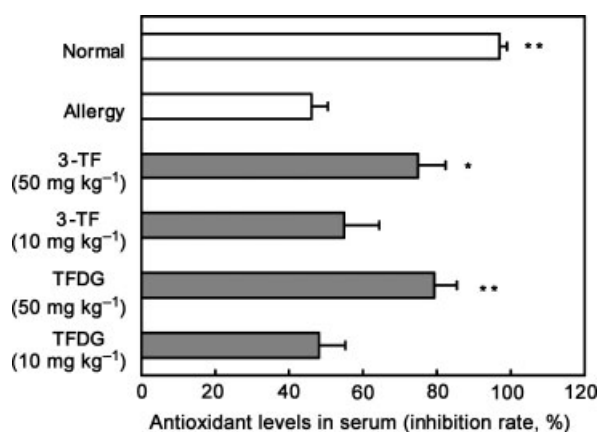


Figure 6. Effects of oral administration of black tea theaflavins on serum antioxidant activities in mice with type IV allergy. Mean $\pm$ SE, $n = 5$. Significant differences from the 'Allergy' group; * $P < 0.05$, ** $P < 0.01$.

of serum antioxidant activities in mice administered theaflavins is thought to contribute to the anti-allergic effects.

CONCLUSIONS

In this study, theaflavins showed a potent preventive effect on oxazolone-induced type IV allergy in male ICR mice, at least in part, via the inhibition of production and/or release of IL-12, IFN- γ and TNF- α in macrophages and/or Th1 cells and the suppression of oxidative stress induced by active oxygen species. The inhibitory effects of theaflavins on the expression of these proinflammatory cytokines would be caused by suppression of some transcription factors such as nuclear factor-kappaB (NF- κ B) in macrophages and/or helper T cells.^{11,22,23} Thearubigin also showed suppressive effects on the expression of NF- κ B in a mouse model of colitis.¹⁰ These inhibitory effects of polyphenols are thought to contribute to the antiallergic effects of black tea. Caffeine is an active ingredient other than catechins, theaflavins and thearubigin for the prevention of type IV allergy.²⁴ Therefore, black tea leaves could be a beneficial food material for type IV allergy.

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