



Continuous Orally Administered Coffee Enhanced the Antigen-Specific Th1 Response and Reduced Allergic Development in a TCR-Transgenic Mice Model

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Coffee is a globally consumed beverage. Although recent studies have suggested that coffee reduced the risk of lifestyle-related diseases, there are few studies regarding allergic response.

This study investigates the effects of orally administered coffee (91 ml/kg/d) on allergic responses using a T cell receptor (TCR)-transgenic DO11.10 mouse allergic model. Splenocytes from coffee-administered naïve mice increased antigen (Ag)-specific interleukin (IL)-12p40 secretion. When Ag sensitization and coffee administration were concurrently performed, the splenocytes from coffee-administered mice showed a decrease of IL-2 and an increase of IL-12p40 secretion. The Ag-specific cutaneous response and serum IgE level were reduced in coffee-administered mice, although, after establishing the allergy, coffee administration did not suppress the allergic reaction.

These results suggest that coffee could induce a Th1-type response of the immune system and prevent an allergy developing. Further studies on the optimum dose, cultivar differences, and roasted degree need to be undertaken.

Key words: allergy; coffee; Th1/2-balance; oral administration

Coffee is a commonly consumed beverage. It is a complex chemical mixture, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolics, with many being formed during the roasting process.¹⁻³ Coffee has been used medicinally since ancient times in the Middle East due to its potential health benefits, and recent studies have suggested that coffee reduced the risk of lifestyle-related diseases such as diabetes, cancer, and heart disease.^{4,5} However, there are few reports regarding the effects of orally administered coffee on immune response and allergy. Some reports have indicated that coffee consumption might trigger the aggravation of inflammatory disorders.^{6,7} Conversely, the immune modulating functions of some compounds in coffee have been reported.^{5,8-11}

We therefore investigated the effects of coffee consumption on immediate-type allergy by using a mouse

allergy model established in our laboratory. This model was transmucosally sensitized with an allergen, as occurs in natural human allergy development. This model had the additional advantage of not requiring an injection of adjuvants, which are not involved in natural allergy induction, thereby avoiding potential problems associated with the route for Ag sensitization. As this model reproduced an immediate onset of the allergic skin reaction with an intradermal injection of the allergen, we could determine the severity of immediate-type allergic diseases with the Ag-specific cutaneous anaphylactic reaction. Hence, we could examine the effects of coffee consumption on allergic responses by using this mouse model.

Materials and Methods

Mice. DO11.10 mice that express T cell receptors (TCR) specific for chicken egg ovalbumin (OVA) 323-339 bound with I-Ad molecules were purchased from Jackson Laboratory (Boston, MA, USA) and maintained in our specific pathogen-free animal facilities. Homozygotes were mated with BALB/c mice to produce the heterozygotes that were used in these experiments. Eight- to 13-week-old mice (both male and female) were used in each study as experimental and control groups. The age, weight and sex of the mice were matched in each experiment. All animal experiments were performed in accordance with the guidelines of the National Food Research Institute for Animal Experiments and law no. 105 and notification no. 6 of the Japanese government. All surgical processes were conducted under anesthesia (0.5% of isoflurane at 1-2 ml/min flow). The mice were kept on a reverse 12-h dark (20:00-8:00)/light (8:00-20:00) cycle and provided with free access to water and diet.

Food and water intake and body weights were monitored at each exchange of diets and water, and mice were observed daily for any signs of ill health. The mice were maintained on the regimens for 2 or 4 weeks and then sacrificed for each experiment.

Chemicals and reagents. RPMI1640, OVA (fraction V), bovine serum albumin (BSA fraction V) and fluorescein isothiocyanate were purchased from Sigma (St. Louis, MO, USA). OVA for oral administration, histamine and formamide were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of the highest purity available from commercial sources.

Coffee administration. The mice were provided with 50% (v/v) sugar-free liquid coffee purchased on the market (Key Coffee®, Japan) *ad libitum* as drinking water for 2 weeks. Deionized water was given as a control.

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Abbreviations: Ag, antigen; APCs, antigen-presenting cells; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; OVA, ovalbumin; TCR, T cell receptor

An intragastric sonde was used for a single oral administration of 100% (v/v) coffee. Deionized water was given as a control.

Antigen sensitization. Ag sensitization was achieved by administering a 2.5% or 5% (w/w) OVA-containing (modified AIN-93) diet (OVA diet; purchased from Oriental Yeast Co., Tokyo, Japan) *ad libitum* for 2 weeks. The OVA diet was administered for the initial 3 d, and the AIN-93 diet was administered for the subsequent 4 d. This dosing regime was conducted twice. After sensitization had been established, the ability to develop an Ag-specific cutaneous anaphylactic reaction was maintained for at least 2 weeks.

Ag-specific vascular permeability assay. As previously described,¹²⁾ the mice were intravenously injected in the tail vein with FITC-BSA (as a tracer) in Tyrode's solution. Immediately afterward, 0.1 and 0.5 μ M of OVA were injected intradermally into the shaved dorsal skin. Tyrode's solution (as a vehicle) and 10 μ g/ml of histamine were always injected as negative and positive controls, respectively. After 30 min, the mice were sacrificed by exsanguination, and the Ag-injected skin samples were punched out. The skin samples were placed directly into the wells of 24-well tissue culture plates, and 1 ml of formamide was added to each. Fifty microliters of mouse serum was added to 0.95 ml of formamide in the same plates. The plates were incubated for 2 h at 50°C. The intensity of vascular permeability was calculated as the value of serum equivalent (μ l) from the fluorescence intensity.

Isolation of immune cells. Single-cell suspensions of splenocytes were prepared from individual mice by mechanical dispersion. The single-cell suspensions of splenocytes were counted and the viability determined by the trypan blue exclusion test. The splenocyte viability of all treatment groups was 95%.

Cell culture. Splenocytes from DO11.10 mice were stimulated with OVA and cultured in RPMI1640-containing 2-mercaptoethanol (5×10^{-5} mol/l), penicillin (100 U/ml), streptomycin (100 μ g/ml), and 10% fetal calf serum (Biowest, Nuaille, France).

ELISA for cytokine production in the culture supernatant. Whole splenocytes were suspended at 1×10^6 cells/well in a 48-well culture plate and stimulated with OVA in a total volume of 500 μ l. The culture supernatant was collected at 48 h (to measure IL-2 and IL-4) and 72 h (for IFN- γ and IL-12p40) after OVA stimulation, and stored at -30°C until needed. The cytokine concentration was measured by using a Mouse ELISA Ready Set Go! Kit (eBioscience, San Diego, CA, USA).

ELISA for the serum antibody titer. Sera were collected 11 and 14 d after starting the experiment and stored at -30°C until needed. The antibody titer was measured by using antibodies from the Mouse Ig ELISA Quantitation Kit (Bethyl, Montgomery, TX, USA). To detect the OVA-specific antibody level in the serum, Maxisorp plates (Nunc, Boston, MA, USA) were coated overnight with OVA (100 μ g/ml) at 4°C. Each serum sample, diluted with the ImmunoBlock (Dainippon Sumitomo Pharma Co., Osaka, Japan), was added to the plate and incubated overnight. Each plate was washed with PBS containing 0.05% Tween 20, before adding HRP labeled anti-mouse Ig, and then incubated for 1 h. A TMB substrate was then added to each plate, and the absorbance was measured (450 nm) after stopping the reaction. To detect of the OVA-specific IgE Ab level in the serum, C96 white Maxisorp plates (Nunc, Boston, MA, USA) were coated with OVA. Each serum sample, diluted with solution 1 of Can Get Signal (Toyobo Biochemicals, Osaka, Japan), was added to the plates and incubated overnight. After each plate had been washed with PBS containing 0.05% Tween 20, HRP-labeled anti-mouse IgE (Bethyl, Montgomery, TX, USA) diluted with solution 1 of Can Get Signal was added, and the plate was incubated for an additional 1 h at room temperature. A chemiluminescent substrate for HRP enzyme (FEM-TOGLOWplus; Michigan Diagnostic LLC, Royal Oak, MI, USA) was added to each plate, and the reaction was measured by using a Wallac 1420 ARVOsx-1 fluoroscan instrument (Perkin Elmer, Wellesley, MA, USA).

Statistical analysis. Data from the experiments with equal concentrations of OVA were compared. A statistical comparison was performed by Student's two-tailed *t*-test; significantly different compared to the control at $p < 0.05$.

Results

Effects of the oral administration of coffee on the body weight of DO11.10 mice

There were no significant differences in portion and beverage consumption between the groups administered with coffee and the control. The mice were provided with 91 ml/kg/d of pure liquid coffee to assess the consumption of coffee and bodyweight. The average body weight gain within the experiments of the control group and coffee-administered groups was 1.47 ± 0.17 g and 1.22 ± 0.37 g, respectively ($N = 6$), there being no significant difference between them.

Effect of the oral administration of coffee on Ag-specific cytokine production in splenocytes isolated from naïve DO11.10 mice

The cytokine concentrations in the culture supernatant of OVA-stimulated splenocytes from naïve DO11.10 mice are shown in Fig. 1. There was no significant difference in Ag-specific IL-2, IL-4, and IFN- γ production between the control groups and those administered with coffee. IFN- γ tended to be higher in the coffee-administered groups ($p < 0.1$). Only IL-12p40 was significantly upregulated in the coffee-administered groups.

Effect of the oral coffee administration on Ag-specific cytokine production in splenocytes isolated from anti-sensitized DO11.10 mice

The cytokine concentrations in the culture supernatant of OVA-stimulated splenocytes from orally sensitized DO11.10 mice are shown in Fig. 2. There was a significant decrease in IL-2 and significant increase in IL-12p40 in the groups administered with coffee, whereas the IFN- γ level did not differ significantly from the control. IL-4 tended to be lower ($p < 0.1$) in the groups administered with coffee, although this was not significant. The IL-5 and IL-10 secretion from splenocytes was not affected in the coffee-administered groups (data not shown).

Effect of the oral coffee administration on serum Ag-specific IgE, IgG1, and IgG2a

To investigate the effect of coffee consumption on the Th2-type immune response, the serum Ag-specific IgE and IgG1, which are typical Th2-type antibodies, and IgG2a (a typical Th1-type antibody) were measured (Fig. 3). In the groups administered with coffee, the IgE titer was significantly lower than that in the control group (Fig. 3A). However, there was no significant difference in IgG1 titer between the coffee-administered groups and the control (Fig. 3B). No difference was apparent in the serum IgG2a titer between the groups administered with coffee and the control (Fig. 3C).

Effect of the oral coffee administration on the Ag-specific cutaneous anaphylactic reaction

To investigate the effect of coffee administration on the allergic reaction, the magnitude of the Ag-specific

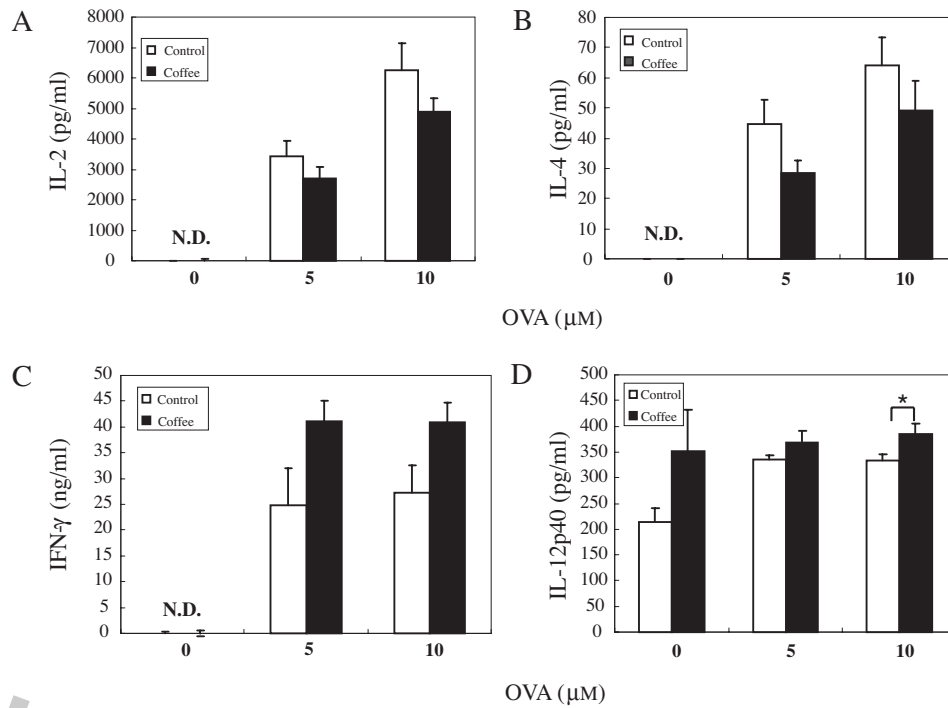


Fig. 1. Effects of an Oral Coffee Administration on the Antigen-Specific Cytokine Production in Splenocytes Isolated from Naïve DO11.10 Mice. DO11.10 mice were administered with coffee or not for the control in the drinking water for 2 weeks *ad libitum*. Mouse splenocytes (1×10^6 cells/well) were stimulated with 5 or 10 μM of OVA. The cytokine concentrations were measured by an ELISA kit. Data are expressed as the mean $\pm$ SE (n = 5). N.D., not detected. *Significantly different from the control ($p < 0.05$). Data shown for splenocytes from the mice administered with coffee (filled columns) and the control mice (unfilled columns) represent the results from four independent experiments.

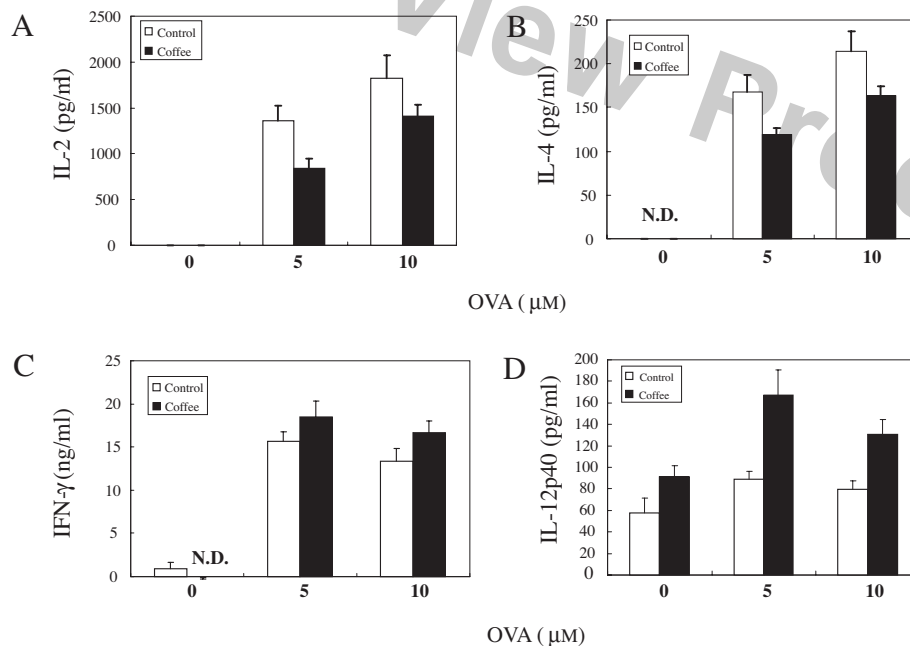


Fig. 2. Effect of an Oral Coffee Administration on the Antigen-Specific Cytokine Production in Splenocytes Isolated from Orally Sensitized DO11.10 Mice.

The mice were administered with coffee or not for the control in the drinking water for 2 weeks *ad libitum*. Oral immunization was conducted in parallel. Mouse splenocytes (1×10^6 cells/well) were stimulated with 5 or 10 μM of OVA. The cytokine concentrations were measured by an ELISA kit. Data are expressed as the mean $\pm$ SE of four to five mice. N.D., not detected. *Significantly different from the control ($p < 0.05$). Data shown for splenocytes from the mice administered with coffee (filled columns) and control mice (unfilled columns) represent the results from four independent experiments.

cutaneous anaphylactic reaction was measured by using the mouse dorsal skin reaction. In these experiments, antigen sensitization was carried out for a 2-week period during which DO11.10 mice were orally administered with coffee. The sensitized DO11.10 mice were injected

intradermally into the dorsal skin with 0.1 μM and 0.5 μM of OVA to provoke a skin reaction. In the coffee-administered groups, Ag-specific plasma leakage was significantly suppressed as compared to the control, and there was no significant difference in the plasma leakage

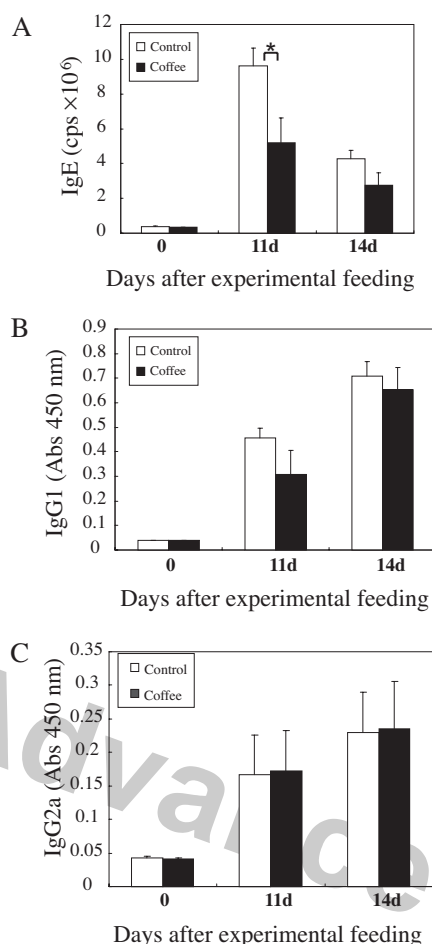


Fig. 3. Serum IgE, IgG1, and IgG2a Titers in DO11.10 Mice Orally Administered with Coffee.

The mice were administered with coffee or not as the control in the drinking water for 2 weeks *ad libitum* while receiving an oral immunization. Antibody (IgG1, IgE and IgG2a) titers were measured by ELISA. Data are expressed in Figs. A and B as the mean $\pm$ SE ($n = 5$), and in Fig. C as the mean $\pm$ SE ($n = 4$). *Significantly different from the control ($p < 0.05$). Data shown for splenocytes from the mice administered with coffee (filled columns) and control mice (unfilled columns) represent the results from four independent experiments.

provoked by histamine between the control and the groups administered with coffee (Fig. 4).

The inhibitory effect of oral coffee administration on the histamine reaction was then investigated. Pure coffee was administered by a single intragastric injection to sensitized mice. After 1 h, the Ag-specific cutaneous anaphylactic reaction was determined (Fig. 5A). In addition, the mice were orally administered with 50% coffee for 2 weeks after 2 weeks of oral sensitization, and the Ag-specific cutaneous anaphylactic reaction was monitored (Fig. 5B). In these experiments, the anaphylactic reactions by Ag and histamine were not altered by the coffee administration. These results indicate that neither the single application nor prolonged administration of coffee affected the Ag-specific cutaneous anaphylactic reaction.

Discussion

We examined the effect of coffee consumption on the systemic immune response and immediate-type allergic reaction. A prolonged coffee intake significantly

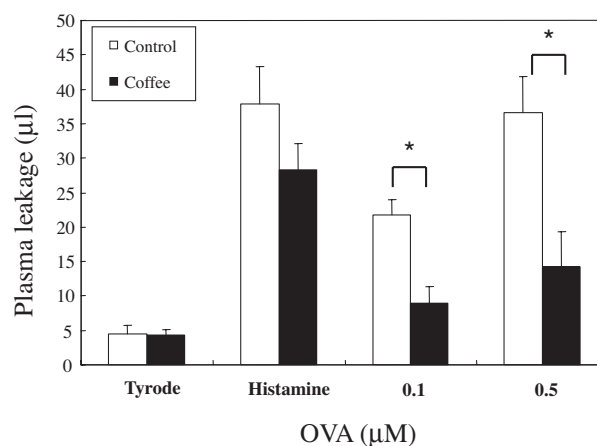


Fig. 4. Antigen-Specific Plasma Leakage in DO11.10 Mice Orally Administered with Coffee.

The mice were administered with coffee or not as the control in the drinking water for 2 weeks *ad libitum* while receiving oral immunization. The mouse dorsal skin was stimulated with 0.1 or 0.5 μ M of OVA and histamine. Plasma leakage at the injection sites was measured by using a fluorescent microplate reader. Data are expressed as the mean $\pm$ SE ($n = 3$). *Significantly different from the control ($p < 0.05$). Data shown for the skin reaction from mice administered with coffee (filled columns) and control mice (unfilled columns) represent the results from three independent experiments.

increased the Ag-specific IL-12p40 secretion in splenocytes isolated from both naïve and sensitized mice (Figs. 1D and 2D). The amount of IL-12p40 secreted, which is a subunit of IL-12, reflects the IL-12 secretion. IL-12 secreted from Ag-presenting cells (APCs), including macrophages and dendritic cells, induces the Th1-type development and inhibits Th2-type development of naïve T cells.

Although not significantly different, the Ag-specific IFN- γ production, which is a typical Th1-type reaction, tended to be higher (Fig. 1C), and Ag-specific IL-4, which is a typical Th2-type cytokine, tended to be lower in splenocytes from the coffee-administered mice (Fig. 2B). IL-4 is a fundamental cytokine inducing an IgE class switch in B cells. Consistent with this, we showed that serum Ag-specific IgE was decreased significantly (Fig. 3A). IL-5 and IL-10 are also Th2-type cytokines whose secretion from splenocytes was not changed in the coffee-administered groups (data not shown).

Mast cells in the skin tissue of sensitized mice must express Fc ϵ IgE receptors on their surface and will bind to Ag-specific IgE. When complexes of the Ag-specific IgE and IgE receptors are cross-linked with specific Ag, mast cells release histamine which increases vascular permeability and provokes a cutaneous anaphylactic reaction. In the mice administered with coffee, the cutaneous anaphylactic reactions provoked by endogenous histamine (released from tissue in an Ag-specific manner) was suppressed, whereas the cutaneous anaphylactic reaction provoked by exogenous histamine (*via* an injection) was not significantly suppressed (Fig. 4).

It was therefore suggested that the anti-allergic effect of coffee was due to the suppression of IgE production, and not to the suppression of histamine action. Additionally, both a prolonged and temporal coffee intake after allergy development did not suppress the Ag-

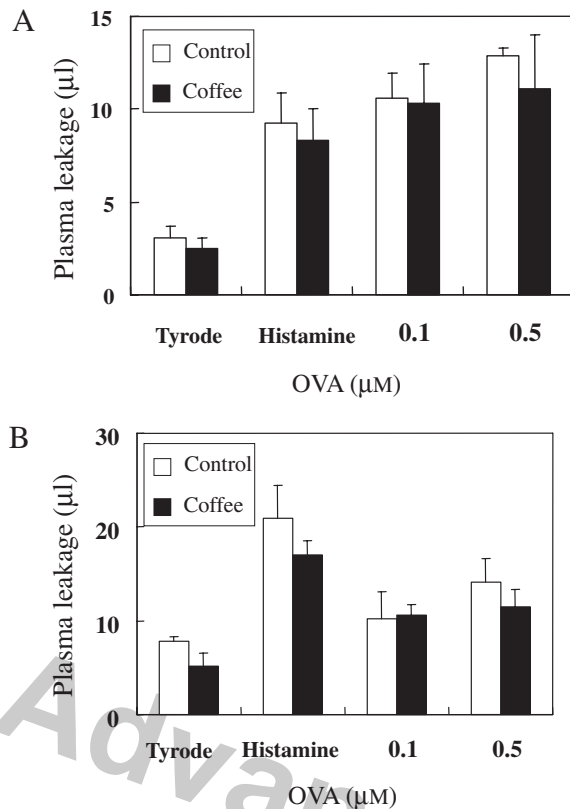


Fig. 5. Antigen-Specific Plasma Leakage in DO11.10 Mice Orally Administered with Coffee after Sensitization.

The mice were administered with coffee or not as the control in the drinking water after an oral sensitization. A single application of a 100% coffee brew was intragastrically administered with a sonde 1 h before measurement (filled columns), and deionized water was given as a control (unfilled columns) in Fig. A. A 50% (v/v) amount of coffee was continuously administered orally for 2 weeks *ad libitum* (filled columns) and deionized water was given as a control (open columns) in Fig. B. The mouse dorsal skin was stimulated with 0.1 or 0.5 µM of OVA and histamine. Plasma leakage at the injection sites was measured with a fluorescent microplate reader. Data are expressed as the mean $\pm$ SE (n = 3).

specific cutaneous anaphylactic reaction (Fig. 5A, and B). These data indicate that coffee intake did not suppress the histamine released from mast cells. It is hence suggested that a prolonged intake of coffee moderated the immediate-type allergic reaction with partial suppression of the Th2 immune response, and not by inhibiting the histamine response. Therefore, a coffee intake might prevent the development of an allergic state, but could not suppress the allergic reaction.

It is well known that a coffee extract includes such chemical compounds as caffeine, caffeic acid, chlorogenic acid, and polysaccharides. It was thought that the immune moderation by a coffee intake might be related to the many compounds in coffee.

We next speculate on the anti-allergic compounds in coffee.

Caffeine is one of the major compounds in coffee, and there are several reports regarding its health benefits. It has also been reported to suppress the lymphocyte function, as indicated by an impaired production of IL-2 and IL-4 through ryanodine receptors on the lymphocytes.^{5,8)} Our study demonstrates that splenocyte Ag-specific IL-2 production in sensitized mice was significantly lower in the mice administered with coffee

(Fig. 2A). Even in the case of splenocytes from naïve mice, the IL-2 and IL-4 production tended to be lower in the coffee-administered groups (Fig. 1A). Hence, caffeine might have downregulated the T cell activities that produce IL-2 and IL-4, and might have led, in part, to a decrease in Ag-specific IgE.

On the other hand, caffeine has little or no effect on IL-12 production.¹³⁾ Our study demonstrates that a continuous oral administration of coffee upregulated the splenocyte Ag-specific IL-12p40 production (Figs. 1D and 2D). IL-12 is produced mainly from APCs and not from T cells.^{14,15)}

Some polysaccharides, like β -glucan, induce APCs to upregulate IL-12 production.^{16–18)} Arabinogalactan is a polysaccharide found in the cell wall of raw and roasted coffee beans.¹⁹⁾ Arabinogalactan, which is likely to be involved in melanoidin formation,²⁰⁾ is the most abundant carbohydrate in brewed coffee.²¹⁾ It has been reported that arabinogalactan from the *Genus Juniperus* induced macrophages to secrete IL-12.²²⁾ Therefore, an oral administration of the arabinogalactan in coffee might upregulated the IL-12 production from APCs and induced a Th1-type immune response, suppressing IgE production and allergic response.

Chlorogenic acid (CGA) is the major polyphenolic antioxidant present in the small molecular fraction of coffee extract. It has been reported that CGA inhibited the histamine release from activated mast cells *in vitro*.¹¹⁾ However, our study indicates that the oral coffee administration had no effect on either the histamine response or histamine release from mast cells. Hence, the histamine-inhibiting release of CGA does not explain the anti-allergic effect of coffee in our studies. In addition, sensitized mice orally administered with CGA have indicated enhanced total IgE in the serum and a high level of IL-4 secreted from mesenteric lymph node cultures.²³⁾ From these reports, orally administered CGA seems to have enhanced the Th2-type immune response. Therefore, CGA in coffee might not be related to the anti-allergic effect of coffee.

It has recently been stated that certain kinds of intestinal microorganisms may be involved in host health. These microorganisms are known as probiotics. It has been demonstrated that several probiotic strains modulate the immune reaction,^{24–26)} and that allergy was suppressed when *Bifidobacterium* and *Lactobacillus* were dominant in the intestines.²⁷⁾ Mannooligosaccharide (MOS) is one of the major oligosaccharides of coffee mannan, and previous studies have confirmed that MOS had a beneficial effect on the intestinal function by improving intestinal microbiota.²⁸⁾ *Bifidobacteria* and *Lactobacilli* can utilize MOS for growth.²⁹⁾ It was recently been reported that dietary MOS reduced Ag-specific IgE in the serum and indicated an anti-allergic effect.³⁰⁾ Therefore, MOS in coffee might be one of the components that suppressed the allergic reaction in our experiment.

In summary, we have shown that the anti-allergic function of coffee might depend on many factors. Therefore, the anti-allergic effect of coffee must be influenced by the cultivar and roasting degree of the coffee beans, and the amount of intake. In this study, the consumption of 50% (v/v) of coffee was no different from that of water; therefore the average amount of

coffee intake was calculated to be 91 ml/kg/d. On the other hand, the water consumption per kg of body weight by mice is approximately three times that of humans. Hence, An adequate amount of coffee for humans needs to be estimated in the future. Our results suggest that coffee would be useful as a prophylactic agent against allergic diseases. A new concept of blended coffee may be important in optimizing the prophylactic effect of coffee on lowering the risk factors for allergy. Future clinical and epidemiological studies may be necessary to determine whether the use of coffee should be influenced by the knowledge of an individual's status of allergic symptoms.

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