

Communication

An *in Vitro* Effect of Coffee on the Antigen-Specific Immune Responses of Naïve Splenocytes

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Received July 27, 2010; Accepted December 21, 2010; Online Publication, February 7, 2011

[doi:10.1271/bbb.100535]

Coffee is a globally consumed beverage with potential health benefits. However, there are few reports about the effects of coffee on immunological functions. We previously reported that in an allergic mouse model, coffee intake prevented allergy development through augmentation of interleukin (IL)-12p40. In order to investigate the anti-allergic activity of coffee, we examined the effect of coffee on antigen (Ag)-specific responses of immune cells *in vitro*. Coffee treatment suppressed proliferation and IL-2 secretion of mouse splenocytes in the same way as splenocytes from mice administered coffee orally. However, IL-12p40 secretion decreased significantly as a result of *in vitro* coffee treatment, which was contrary to the results obtained from experiments of mice administered coffee orally. Therefore, modification associated with oral administration might influence the anti-allergic activity of coffee.

Key words: coffee; Th1; Th2; naïve immune cells; anti-allergy

Coffee is known to have potential health benefits, and recent studies have suggested that coffee consumption reduces the risk of certain chronic diseases.^{1,2} Coffee contains various compounds, such as carbohydrates, lipids, nitrogenous substances, vitamins, minerals, alkaloids, and phenolics. In addition to these constituents, other compounds are produced during the roasting process,^{3,4} some of which have been shown to modulate immune responses *in vitro*.^{5–8} However, there are few reports regarding the potential anti-allergic activity of coffee *in vivo*. Using an allergic mouse model developed in our laboratory, we found that orally administered coffee induced Th1-type immune reactions and prevented allergy development,⁹ though the active center of the immuno-modulative effect of coffee was not clear.

The present study was conducted to investigate the immuno-modulatory components of coffee using naïve immune cells from DO11.10 mice which express the T cell receptor (TCR) specific for OVA. DO11.10 mice were purchased from Jackson Laboratory (Boston, MA) and were maintained in our specific pathogen-free animal facilities. The animal studies were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization (NARO), Japan.

The medium used for cell culture was RPMI 1640 (Sigma, St. Louis, MO), containing 2-mercaptoethanol (5×10^{-5} mol/L), penicillin (100 U/mL), streptomycin (100 µg/mL), and 10% fetal calf serum (Biowest, Nuaille, France). Commercially provided freeze-dried coffee (Nestle, Tokyo) is used as hot-water extract of coffee. It was dissolved in distilled water at a concentration of 10 mg/mL and was stored at -30°C as a stock solution after autoclave sterilization. The coffee stock solution was diluted with culture medium immediately before use.

Single cell suspensions of splenocytes were prepared from individual DO11.10 mice by mechanical dispersion, and were suspended at 1×10^6 cells/mL with culture medium in 96-well culture plates (Nunc, Boston, MA). Splenocytes were treated with 6.25, 25, or 100 µg/mL of coffee and 5 or 10 µM of intact OVA (fraction V grade, Sigma) for up to 72 h. Prior to the experiments, we checked that these concentrations of coffee did not exhibit acute toxicity to splenocytes (data not shown).

An Ag-specific cell proliferation assay was performed in a total volume of 100 µL per well. Cell proliferation was measured using a Cell Proliferation ELISA, BrdU (chemiluminescent) kit (Roche Molecular Biochemicals, Basel, Switzerland) 72 h after OVA stimulation. An Ag-specific cytokine secretion assay was performed in a total volume of 300 µL per well. Culture supernatants were collected at 48 h for measurement of IL-2, IL-4, and IL-5 and at 72 h for measurement of IL-10, IL-12p40, and IFN- γ after OVA stimulation. Supernatants were stored at -30°C until use. Cytokines were measured using Mouse Cytokine ELISA Ready Set Go! kits (eBioscience, San Diego). Dendritic cells and CD4⁺ T cells were prepared from pooled splenocytes from four DO11.10 mice by the MACS[®] system (Miltenyi Biotec, Bergish Gladbach, Germany). CD4⁺ T cells (1×10^6 cells/mL) separated with anti-mouse CD4 labeled MACS microbeads and dendritic cells (3×10^6 cells/mL) separated with anti-mouse CD11c labeled MACS microbeads were co-cultured for 72 h in the presence of 5 µM of OVA and 100 µg/mL of coffee in a total volume of 150 µL per well. Culture supernatants were collected for measurement of IL-12p40.

The effects of various concentrations of coffee on cell proliferation were compared for each concentration of

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Abbreviations: Ag, antigen; IL, interleukin; OVA, chicken ovalbumin; TCR, T cell receptor

OVA tested by ANOVA with Dunnett's multiple comparison of means test.

In vitro coffee treatment suppressed Ag-specific proliferation of splenocytes significantly (Fig. 1). The effects of coffee treatment on the secretion of Ag-specific Th1-type cytokines (IL-2, IL-12p40, and IFN- γ) and Th2-type cytokines (IL-4, IL-5, and IL-10) by splenocytes is shown in Fig. 2. Coffee treatment significantly reduced Ag-specific IL-2 and IL-12p40 secretion and tended to reduce the secretion of IFN- γ (Fig. 2A–C). There was no effect on the secretion of Th2-type cytokines (Fig. 2D–F). We previously reported that Ag-specific proliferation and IL-2 secretion of splenocytes from mice administered coffee orally decreased compared to those of control mice, while secretion of Th2-type cytokines did not change.⁹⁾ Caffeine has been reported to reduce IL-2 secretion by immune cells.^{6,10)} IL-2 is the most important cytokine with respect to Ag-specific T cell proliferation. Hence, the caffeine in coffee might contribute to the suppression of splenocyte proliferation through a decrease in IL-2 secretion.

On the other hand, secretion of Ag-specific IL-12p40 and IFN- γ in the present study appeared inconsistent

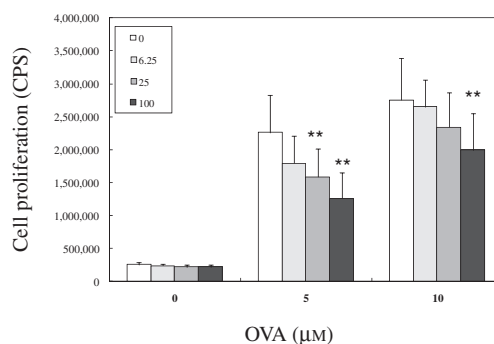


Fig. 1. Proliferation of Splenocytes from Naïve Transgenic Mice with Specific-Antigen in the Presence of Coffee.

Splenocytes from DO11.10 mice (1×10^5 cells/well) were treated with 0 (control), 6.25, 25, or 100 $\mu\text{g/mL}$ of coffee and 5 or 10 μM of OVA for up to 72 h. Cell proliferation for duplicate cultures was measured by cell proliferation ELISA and BrdU (chemiluminescent) kit. Data are expressed as mean $\pm$ SE of four mice for one of three independent experiments. Comparisons were made among equal OVA concentrations. Statistical comparisons were carried out by ANOVA with Dunnett's multiple comparison of means test. Comparison with control showed significant difference (** $p \leq 0.01$).

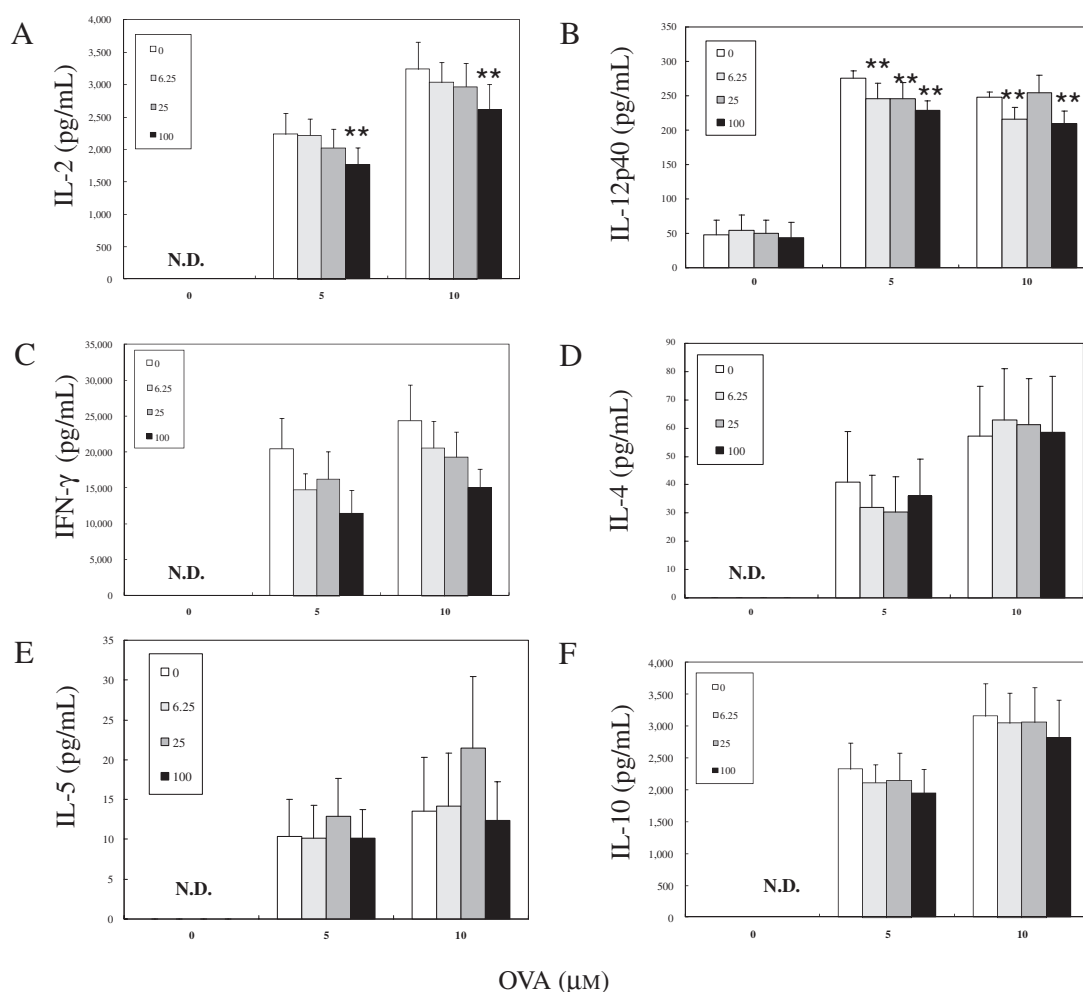


Fig. 2. Effects of Coffee on Antigen-Specific Th1-Type Cytokine (A, IL-2; B, IFN- γ ; C, IL-12p40) and Th2-Type Cytokine (D, IL-4; E, IL-5; F, IL-10) Production by Splenocytes from Naïve Mice.

Splenocytes (3×10^5 cells/well) were cultured with 0 (control), 6.25, 25, or 100 $\mu\text{g/mL}$ of coffee in the presence of 5 or 10 μM of OVA in 96-well plates for 72 h. Cytokine concentrations were measured using Mouse ELISA Ready Set Go! kits. Data are expressed as the mean $\pm$ SE of four mice for one of three independent experiments. Comparisons were made among equal OVA concentrations. Statistical comparisons were carried out by ANOVA with Dunnett's multiple comparison of means test. Comparison with control showed significant difference (** $p \leq 0.01$).

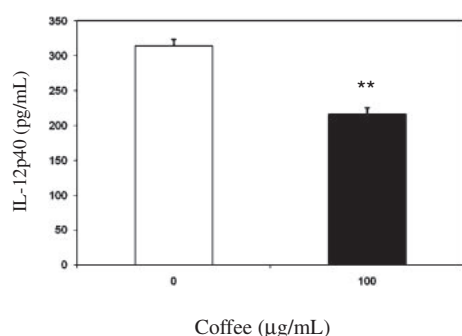


Fig. 3. Effects of Coffee on Antigen-Specific IL-12p40 Production of Splenic Dendritic Cells from Naïve Transgenic Mice.

Splenic CD11c⁺ cells (1.125×10^5 cells/well) and CD4⁺ cells (0.375×10^5 cells/well) were co-cultured with 0 (control) or 100 µg/mL of coffee in the presence of 5 µM of OVA in 96-well plates for 72 h. IL-12p40 concentrations were measured using a Mouse ELISA Ready Set Go! kit. Data are expressed as the mean $\pm$ SE from triplicate cultures for one of two independent experiments. Statistical comparisons were carried out using Student's two-tailed *t* test. Comparison with control showed significant difference (***p* $\leq$ 0.01).

with our previous *in vivo* results. Secretion of IL-12p40 and IFN- γ increased in splenocytes from mice administered coffee orally. IL-12p40 is a subunit of bioactive IL-12 (IL-12p70), which is an essential inducer of Th1-type T cells, which prevent allergy development,¹¹⁾ though IL-12p70 was too small in amount to detect in our culture system (data not shown). We concluded that orally administered coffee induces a Th1-type immune reaction, preventing allergy development through enhancement of IL-12 secretion in this allergic mouse model. However, the major components of coffee, caffeine and chlorogenic acid, have been reported to have little or no effect on IL-12 secretion.^{12,13)}

Arabinogalactan is one of the major polysaccharides found in brewed coffee, and it has been reported that arabinogalactan from juniper (*Juniperus scopolorum*) enhances IL-12 secretion.¹⁴⁾ Hence we speculated that arabinogalactan in brewed coffee increased IL-12 secretion by splenocytes from the mice administered coffee orally. However, direct exposure of coffee to splenocytes reduced IL-12p40 secretion significantly (Fig. 2B).

Dendritic cells are antigen-presenting cells that can stimulate naïve T cells. IL-12 secreted from dendritic cells plays important roles in the development of Th1-type T cells, and IL-12 secretion from dendritic cells is upregulated with stimulation from activated T cells. Coffee treatment suppressed IL-12p40 secretion from CD11c⁺ dendritic cells co-cultured with Ag-stimulated T cells significantly (Fig. 3). These results suggest that directly applied coffee suppressed IL-12 secretion, contrary to oral administration.

Most of the arabinogalactan in coffee is incorporated by ionic bonds into negatively charged melanoidins.¹⁵⁾ Recently, it was reported that the antioxidative capacity of melanoidins is enhanced by gastrointestinal enzymatic digestion, presumably because compounds ionically bound to the melanoidin skeleton are enzymatically released.¹⁶⁾ These results suggest that gastrointestinal enzymatic digestion is required to elicit the anti-allergic activity of coffee arabinogalactan.

Our data suggest that coffee contains ingredients that suppress IL-12 secretion and induce anti-allergic activities. Our results suggest that coffee's effect on the immune system is attributable to multiple factors that are influenced by the species and roast level of the coffee beans. The reasons for the contrary effects of coffee compounds *in vivo* are unclear. Further studies must be conducted to identify the compounds involved in order to elucidate the anti-allergic activity of coffee.

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