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**Rutinosylated ferulic acid attenuates food allergic response and colitis
by up-regulating regulatory T cells in mouse models**

Shigeru Katayama ^{†,§}, Fumiaki Ohno [†], Takakazu Mitani ^{†,§}, Hiroshi Akiyama [#], and
Soichiro Nakamura ^{†,*}

[†] Department of Bioscience and Biotechnology, Shinshu University, 8304

Minamiminowa, Ina, Nagano 399-4598, Japan

[§] Interdisciplinary Cluster for Cutting Edge Research, Shinshu University, 8304

Minamiminowa, Ina, Nagano 399-4598, Japan

[#] National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya, Tokyo 158-8501,

Japan

Corresponding author:

Soichiro Nakamura, PhD

Department of Bioscience and Biotechnology, Shinshu University, 8304

Minamiminowamura, Ina, Nagano 399-4598, Japan

Tel&FAX: +81-265-77-1609, e-mail: snakamu@shinshu-u.ac.jp

Abstract

The purpose of this study was to screen phytochemicals capable of inducing immune tolerance via enhanced TGF- β 1 secretion and investigate their effects in a mouse model of food allergy and colitis. In a screening test using THP-1-derived dendritic cells, a significant increase in TGF- β 1 levels was observed upon treatment with ferulic acid (FA) and its glycosides, among which FA rutinoside (FAR) induced the highest level of TGF- β 1 secretion. Oral administration of FAR suppressed serum levels of IgE and histamine in ovalbumin-sensitized mice and triggered the differentiation of regulatory T (Treg) cells. Compared with the control, FAR treatment also induced stronger TGF- β 1 secretion from splenic dendritic cells. FAR treatment attenuated dextran sulfate sodium-induced colitis in model mice and induced Treg differentiation. These results suggest that FAR exerts potent immunomodulatory effects against allergic and intestinal inflammatory responses by inducing Treg differentiation.

Keywords: dendritic cells, ferulic acid, glycoside, immune tolerance, regulatory T cells

37 **Introduction**

38 Immunotherapy, which entails gradually increasing the exposure to allergens
39 with the aim of desensitization and promoting tolerance, is the most researched
40 approach to treating allergies ¹. Currently, oral immunotherapy appears to be more
41 promising than subcutaneous immunotherapy for treating food allergies because the
42 subcutaneous approach results in unacceptably high rates of severe adverse
43 reactions.^{2,3} However, even oral immunotherapy poses a high risk of IgE-mediated side
44 effects, and the establishment of more effective and safe immunotherapeutic strategies
45 has been long awaited.

46 Some phytochemicals have been reported to show immunomodulatory effects,
47 such as the regulation of the Th1/Th2 balance and enhanced IgA production in the
48 gut.⁴ Generally, the bioactivity of a phytochemical depends on its structure,
49 particularly the numbers and positions of hydroxyl groups in relation to the carboxyl
50 functional group.⁵ Numerous attempts have been made to modify phytochemicals for
51 improvement of their functional properties. Multiple studies have confirmed that the
52 biological activities of phenolic acids can be improved by lipophilization^{6,7} and
53 glycosylation.^{8,9} As glycosylation reinforce the hydrophilic nature by conjugation of
54 sugars, glycosylation of hydrophilic compounds can be a potent strategy to alter not

55 only their solubility and cell penetrability but also their biological activity.

56 Bioactive compounds with regulating immunomodulatory cytokines might offer
57 a good strategy for development of immunomodulatory agents. In Particular,
58 immunosuppressive cytokines, such as TGF- β and IL-10, play roles in the induction
59 and maintenance of regulatory T (Treg) cells, which are critical for oral
60 immunotherapies.^{10,11} Treg induction mainly depends on the interaction of T cells with
61 tolerogenic dendritic cells (DCs), and mature DCs induce the expansion of Treg cells
62 together with secreted cytokines, including TGF- β .¹¹ Therefore, phytochemicals with
63 TGF- β -inducing activity might be immunomodulatory agents with potent action
64 against immune diseases, such as food allergy and inflammatory bowel disease (IBD).

65 In this study, we screened for phytochemicals having immunomodulatory effects
66 using a dendritic cell-based assay system, structurally modified the obtained candidates
67 to improve their functionality, and evaluated their immunomodulatory effects in a
68 mouse model of food allergy symptoms and IBD.

69 **Materials and methods**

70 **Materials**

71 The naturally occurring phytochemicals rutin and ferulic acid (FA) were
72 purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). RPMI1640 was
73 obtained from Nacalai Tesque (Kyoto, Japan). Recombinant human IL-4 was from
74 PeproTech (Rocky Hill, NJ, USA), and phorbol 12-myristate 13-acetate (PMA) was
75 from Sigma (St. Louis, MO, USA). Other reagents used for flow-cytometric analysis
76 were purchased from BD Biosciences (San Jose, CA, USA). All other reagents were of
77 biochemical or HPLC grade.

78

79 **Enzymatic synthesis of FA glycosides**

80 Phenolic acid glycosides of FA were enzymatically synthesized according to a
81 previously described method ⁸. Briefly, for the preparation of FA glucoside (FAG), the
82 reaction mixture comprised 10 mM quercetin 3-glucopyranoside, 3.75% (w/w of
83 quercetin 3-glucopyranoside substrate) rutinase, and 10 mM FA in 1 mL of 20 mM
84 acetate buffer (pH 5.0). For preparation of FA rutinoside (FAR), the reaction mixture
85 comprised 10 mM rutin, 3.75% (w/w of rutin substrate) rutinase, and 10 mM FA in 1
86 mL of 20 mM acetate buffer (pH 5.0). The tubes were incubated at 40°C for 24 h on a

thermomixer (Eppendorf, Hamburg, Germany) with shaking at 1,300 rpm. Then, the mixtures were centrifuged at 15,000 $\times$ g for 30 min at 4°C, after which the supernatant was passed through a 0.45- μ m syringe filter and applied to an HPLC column (Inertsil NH₂ column, 4.6 $\times$ 250 mm; GL Sciences, Tokyo, Japan). Elution was performed with 75% acetonitrile at a flow rate of 1 mL/min, and the fractionated compounds were detected using an RI detector (RI-1530; Jasco, Tokyo, Japan) and a UV detector (UV-2075; Jasco) set at 280 nm. The column temperature was set at 30°C. The synthesized glycosides were collected, the acetonitrile was evaporated, and the residue was freeze-dried.

Assay of THP-1-derived dendritic cells (TDDCs)

The human acute monocytic leukemia cell line THP-1 was obtained from JCRB Cell Bank (Osaka, Japan) and maintained in RPMI 1640 medium supplemented with 10% FBS, 50 U/mL penicillin, and 50 μ g/mL streptomycin (Wako, Osaka, Japan) at 37°C in a humidified atmosphere of air with 5% CO₂. THP-1 were differentiated to DCs according to a previously reported method.¹² THP-1 was seeded at 5 $\times$ 10⁵ cells/well in a six-well plate and incubated in 2 mL/well of RPMI 1640 medium supplemented with PMA (final concentration; 20 ng/mL) and IL-4 (20 ng/mL). On day

4, the immature DCs were washed with PBS and incubated for 72 h in the absence or presence of 17 phytochemical samples (50 μ M) in RPMI 1640 medium supplemented with 10% FBS, 50 U/mL penicillin, and 50 μ g/mL streptomycin. TGF- β 1 in the culture supernatant was quantified using a commercial ELISA kit (Enzo Life Sciences, Plymouth Meeting, PA, USA). Differentiation of THP-1 to DCs was confirmed by marker protein expression of CD11c⁺.

111

112 **Animals**

BALB/c mice (female, 5 weeks old) were purchased from Charles River Ltd. (Tokyo, Japan) and acclimatized for 1 week before the start of the study. The mice were provided free access to a commercial pellet diet (MF; Oriental Yeast Co., Tokyo, Japan) and water. The animal room was maintained at constant temperature (22 $\pm$ 2°C) and humidity (55% $\pm$ 10%) with a 12-h light/dark cycle. The mice were treated according to the institutional guidelines for animal experimentation at Shinshu University.

120

121 **OVA-sensitization and treatment**

In this experiment, the mice were divided into 4 groups (6 mice per group): a

control group, an OVA group, an OVA+FA group, and an OVA+ FAR group. For the OVA, OVA+FA, and OVA+FAR groups, the mice were immunized with 50 μ g of OVA dissolved in 100 μ L of PBS mixed with an equal volume of alum adjuvant by intraperitoneal injection. The mice received a second-immunization with an intraperitoneal injection of 25 μ g of OVA and alum adjuvant 14 days after the initial immunization. Seven days after the second immunization, the mice were fed homemade feed containing 50 mg of FA or FAR per 100 g of MF pellet ad libitum. Thereafter, oral feeding was continued for 28 days, and oral challenge was conducted with 50 mg of OVA on days 21 and 24 of sample feeding. Control mice were also given the commercial pellet diet on the same schedule without immunization. The mice were sacrificed by CO₂ asphyxiation, and the serum and spleen were collected from each mouse.

135

136 **Assessment of hypersensitivity reactions**

137 Anaphylactic symptoms were evaluated 40 min after challenge dose by using a
138 scoring system as described by Li et al.¹³ Scores were as follows: 0, no symptoms; 1,
139 scratching and rubbing around the nose and head; 2, puffiness around the eyes and
140 mouth, diarrhea, piloerection, reduced activity, and/or decreased activity with increased

141 respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and
142 the tail; 4, no activity after prodding or tremor and convulsion; and 5, death.

143

144 **Measurement of total IgE, specific IgE, and total IgA antibodies**

145 The serum histamine concentration was determined using a histamine EIA kit
146 (SPI-Bio, Bretonneux, France). The levels of total IgE, specific IgE, and total IgA in
147 the sera were measured by ELISA with horseradish peroxidase (HRP)-labeled
148 antibodies and hydrogen peroxide with o-phenylenediamine as the substrate.
149 Anti-mouse IgE and IgA antibodies and HRP-conjugated anti-mouse IgE and IgA were
150 used, all of which were purchased from Pierce (Rockford, IL, USA).

151

152 **Flow-cytometric analysis of Treg cells**

153 Flow cytometry was used to determine the differentiation level of Treg cells.
154 Cells isolated from the collected spleens were stained with FITC-labeled anti-CD4
155 (1:100; BD Bioscience, San Jose, CA, USA) and PE-labeled anti-CD25 (1:100; BD
156 Bioscience). After surface staining, the cells were washed, fixed, permeabilized, and
157 stained for intracellular Foxp3 by using Alexa Fluor 647-labeled anti-Foxp3 (1:100;
158 BD Biosciences) monoclonal antibodies and a mouse Foxp3 buffer set (BD

159 Biosciences) according to the manufacturer's instructions for Treg analysis. The cells
160 were analyzed using a FACSCalibur flow cytometer with CellQuest software (BD
161 Biosciences).

162

163 **Determination of TGF- β 1 levels produced from CD11c⁺ cells**

164 For separation of CD11c⁺ cells from the isolated spleen cells, a suspension of
165 spleen cells was added to 500 μ L of MACS buffer, mixed with 10 μ L of anti-CD11c
166 microbeads (Miltenyi Biotec, Gladbach, Germany), incubated for 15 min in the dark at
167 4°C, and centrifuged at $200 \times g$ for 10 min. The supernatant was aspirated completely,
168 and the cells were resuspended in 500 μ L of MACS buffer. An MS column adapter was
169 inserted into the magnetic field of a Mini MACS separator. The column was washed
170 three times with 3 mL of MACS buffer and then removed from the magnetic field.
171 MACS buffer (1 mL) was then pipetted into the column to flush out the cell suspension.
172 The cells obtained, regarded as CD11c⁺ cells isolated from spleen, were suspended (1
173 $\times 10^5$ cells/well) in RPMI 1640 medium supplemented with 10% FBS, 50 U/mL
174 penicillin, and 50 μ g/mL streptomycin, and further incubated for 72 h with PBS, or
175 100 μ g/mL FA or FAR. The TGF- β 1 concentration of the culture supernatant was
176 measured using a commercial ELISA kit (Enzo Life Sciences).

177

178 Induction of dextran sulfate sodium colitis

179 In this experiment, mice were divided into 4 groups (6 mice per group): a control
180 group, a dextran sulfate sodium (DSS) group, a DSS+FA group, and a DSS+FAR
181 group. For the DSS, DSS+FA, and DSS+FAR groups, DSS was orally administered to
182 induce colitis. Control mice received sterile water during the study period. The animals
183 were fed homemade pellets containing 0.05% (w/w) FA or FAR ad libitum for 21 days.
184 After 7 days of exposure to 2% (w/v) DSS from day 14 to day 21, the mice were
185 sacrificed following CO₂ inhalation. Their colons were excised from the cecum to the
186 pelvic brim, and colon length was measured in centimeters. Each colon was equally
187 divided into proximal and distal colon and used for RNA isolation and histology. After
188 colon excision, a portion of the distal colon was fixed in 10% neutral formalin buffer
189 (Sigma). Paraffin sections (5 µm thick) were cut transversely and stained with
190 hematoxylin and eosin (H&E).

191

192 Assessment of clinical score

193 The clinical score was assessed on day 22 by using the scoring system described
194 by Chassaing et al..¹⁴ Briefly, normal stool consistency with negative hemocult was

195 scored as 0, soft stools with positive hemocult as 1; very soft stools with traces of
196 blood as 2; and watery stools with visible rectal bleeding as 3.

197

198 **Real-time PCR**

199 Total RNA was isolated from the proximal colon using RNAiso Plus (TaKaRa
200 Bio, Shiga, Japan), and mRNA was reverse-transcribed with a ReverTra Ace kit
201 (Toyobo, Osaka, Japan). The cDNA was quantified with the KAPA SYBR FAST
202 Master Mix kit (Kapa Biosystems, Woburn, MA, USA) on a Dice Real-Time System
203 thermal cycler (TaKaRa Bio). Mouse primers used in the analysis were as follows:
204 β -actin, 5'-ATCATTGCTCTCCTGAGCG-3' and 5'-GCTGATCCACATCTGGAA-3';
205 IL-17, 5'-GCTGATCCACATCTGGAA-3' and 5'-GCTGATCCACATCTGGAA-3';
206 and TNF- α , 5'-GCTGATCCACATCTGGAA-3' and
207 5'-GCTGATCCACATCTGGAA-3'. Target gene expression was normalized to β -actin
208 expression.

209

210 **Statistical analysis**

211 Data are presented as the mean $\pm$ SD. Means were compared using Student's t-test;
212 $p < 0.05$ was considered significant.

213

214 **Results**215 **Immunomodulatory effect assay using TDDCs**

216 We first screened phytochemicals having immunomodulatory effects on the basis
217 of TGF- β 1 secretion from DCs. TGF- β 1 is a well-known immunosuppressive cytokine
218 that induces differentiation of Treg cells.¹⁰ TDDCs were incubated with different
219 phytochemicals at a concentration of 50 μ M for 3 days, and TGF- β levels in the culture
220 supernatant were determined by ELISA. There were no significant changes in cell
221 viability in response to treat with the samples used in this study (data not shown). As
222 shown in Figure 1, 3 phytochemicals, namely, quercetin, curcumin, and kaempferol,
223 did not alter the TGF- β 1 levels as compared to the control. Six other phytochemicals,
224 epicatechin, rosmarinic acid, vanillic acid, sinapic acid, isoquercetin, and caffeic acid,
225 significantly decreased TGF- β 1 secretion levels, whereas hydroquinone, catechin,
226 chlorogenic acid, and capsaicin showed slight decreases. In contrast, p-coumaric acid,
227 isoferulic acid, and rutin increased TGF- β 1 secretion slightly but not significantly,
228 whereas a significant increase was observed for FA treatment. We also assessed the
229 effects of enzymatically synthesized FAG and FAR on TGF- β secretion in the TDDCs.
230 A further increase was observed for FAR treatment. These results suggest that FA and

231 FAR are potent immunomodulatory agents, with the potential to induce Treg cells and
232 suppress excessive immune reactions such as allergic symptoms and IBD.

233

234 **Immunomodulatory effect of FAR**

235 We next investigated the immunomodulatory effects of FA and FAR in
236 OVA-sensitized mice as a mouse model of egg allergy (Figure 2A). Compared with
237 OVA-sensitized mice, mice administered with FA and FAR for 28 days developed less
238 hypersensitivity; in particular, FAR-fed mice exhibited a significant reduction (Figure
239 2B). Oral administration of FA and FAR also decreased the serum levels of histamine,
240 total IgE, and OVA-specific IgE as compared to OVA treatment (Figures 2C, 2D, and
241 2E). In contrast, total IgA levels in the FA- and FAR-treated groups were higher than
242 that those in the group treated with OVA alone (Figure 2F). The population of Treg
243 cells in the OVA-treated group was smaller than that in the control group (Figures 3A
244 and 3B). Treatment with FAR increased the population of Treg cells as compared to the
245 group treated with OVA alone, whereas FA treatment induced a population similar to
246 that in the OVA-alone group. We further investigated whether the increased Treg
247 population was caused by TGF- β secretion from DCs. CD11c⁺ cells isolated from
248 spleen cells were incubated with FA or FAR, and the TGF- β 1 level of the culture

249 supernatant was determined by ELISA. As shown in Figure 3C, the TGF- β 1 level was
250 increased by treatment with FAR as compared to the control, whereas the TGF- β 1 level
251 remained unchanged by treatment with FA.

252

253 **Anti-inflammatory effect of FAR**

254 IBD is defined as a group of chronic inflammatory disorders of the colon and/or
255 small intestine.¹⁵ We further investigated the immunomodulatory effect of FAR in
256 DSS-treated mice as a mouse model of IBD (Figure 4A). DSS intake induced a
257 significant decrease in body weight, whereas treatment with FA and FAR suppressed
258 this effect (Figure 4B). Similar results were observed for colon length: FA and FAR
259 suppressed the reduction in colon length induced by DSS (Figure 4C). In addition,
260 DSS intake resulted in an increase in clinical score, whereas treatment with FA and
261 FAR showed lower levels compared to treatment with DSS alone (Figure 4D). Colonic
262 histology revealed epithelial disruption upon DSS treatment, whereas FA and FAR
263 treatments led to recovery of the epithelial disruption (Figure 4E). We also investigated
264 whether the immunosuppressive effects of FA and FAR resulted from the
265 differentiation of Treg cells. As shown in Figures 5A and 5B, FAR treatments
266 significantly increased the Treg population in the spleen as compared to treatment with

267 DSS alone, whereas the Treg population remained unchanged after FA treatment.
268 Marked decreases in the gene expression of the inflammatory cytokines IL-17 and
269 TNF- α were observed in the colon tissues of FA- and FAR-treated groups; in particular,
270 FAR-fed mice showed a highly significant decrease in the IL-17 level (Figures 5C and
271 5D).

272

273 Discussion

274 The incidence of autoimmune and autoinflammatory disorders is rapidly
275 increasing in developed countries.¹⁶ Various studies have suggested Treg cell-mediated
276 immune suppression to be a potential therapeutic approach.¹⁷⁻¹⁹ In this study, we
277 demonstrated the ability of FAR to induce Treg cell differentiation. In mouse models of
278 both egg allergy and IBD, FAR showed potent immunosuppressive effects by
279 increasing the Treg cell population. FA also suppressed the allergic reaction and
280 attenuated the intestinal inflammation similarly to FAR; however, no significant
281 induction in differentiation of Treg cells was observed in the FA-fed group. FA belongs
282 to the family of phenolic acids and is highly abundant in fruits and vegetables. Several
283 studies have shown that FA acts as a potent antioxidant by scavenging free radicals and
284 enhancing the cellular stress response.²⁰ In addition, derivatives of FA have been

285 reported to act as anti-inflammatory agents. For example, Islam et al. showed that a
286 mixture of phytosteryl ferulates isolated from rice bran can attenuate DSS-induced
287 colitis in mice, and that this might be mediated by inhibition of NF-kappaB (NF-kB)
288 activity.²¹ Intact FA suppresses inflammation; however, its effect is weaker than those
289 of phytosteryl ferulates. FA ethyl ester has also been shown to exert anti-inflammatory
290 activity by inhibiting leukocytes, pro-inflammatory cytokines, and oxidative stress in a
291 rat model of arthritis.²² The FA moiety in these FA derivatives might be responsible for
292 these immunomodulatory effects. Additionally, Lee et al. have demonstrated that FA
293 induces a Th1 response by modulating DC function and ameliorates Th2-mediated
294 allergic airway inflammation by restoring Th1/Th2 imbalance.²³ The results in the
295 present study corroborated the anti-allergic effect of FA and FAR; however, FA did not
296 stimulate the induction of Treg differentiation to the extent that FAR did. These
297 findings suggest that the effects of intact FA were mediated by inhibition of NF-kB
298 activity and restoration of Th1/Th2 imbalance, and that rutinosylation of FA enables
299 induction of Treg differentiation.

300 After ingestion, neither FA nor 5-O-feruloyl-l-arabinofuranose is degraded by
301 the acid environment in the stomach, and both remain stable throughout intestinal
302 transit.²⁴ In the colon, bound FA is released from parent compounds by microbial

303 cinnamoyl esterase,²⁵ xylanase, and FA esterase,²⁶ and it is mainly absorbed by passive
304 diffusion (~90%), whereas only a small percentage is actively transported via the
305 monocarboxylic acid transporter.²⁷ Glycosides of FA, such as FAR, might be degraded
306 by microbial enzymes in the colon; however, the Treg-inducible effect of FAR was
307 found to be higher than that of FA. In the allergic mice, DCs in Peyer's patch cells
308 might have recognized the invading compounds or antigens and transmitted the
309 information throughout the body. Thus, intact FAR could directly affect the DCs in the
310 intestine and induce the differentiation of Treg cells.

311 Astilbin, a flavanone glycoside, has (+)-taxifolin substituted by an
312 α -L-rhamnosyl moiety at position 3 via a glycosidic linkage. Ding et al. have
313 demonstrated that administration of astilbin resulted in attenuated severity of colitis
314 and enhanced production of Treg cells, which was caused by induction of TGF- β and
315 IL-10 production in DCs.²⁸ In our study, the TGF- β -inducing effect of FAR was higher
316 than that of FAG, suggesting that the rhamnosyl moiety is a major active site. Rutin, a
317 quercetin rhamnoside, increased the production of TGF- β 1, whereas quercetin did not.
318 The aglycone moiety might have contributed to the induction of TGF- β production. On
319 th, TGF- β induction has been reported to depend on the Toll-like receptor 2 (TLR2)
320 and ERK-AP-1 kinase pathways.^{29,30} Although it remains unclear whether the

321 rhamnosyl moiety affects the TLR2-ERK-AP-1 pathway, further investigation of the
322 detailed molecular mechanism is necessary.

323 Our results demonstrate that orally administered FAR has anti-allergic and
324 anti-inflammatory effects via enhanced Treg induction. Based on their cytokine
325 secretion profile and effector function, CD4⁺ T lymphocytes can be divided into Th1,
326 Th2, Th17, and Treg cell subsets.³¹ A recent study reported that the function of effector
327 T cells, such as Th1, Th2, and Th17 cells, is regulated by Treg cells.³²
328 Immunosuppressive Treg cells play important roles in the maintenance of immune
329 homeostasis and induction of immune tolerance by producing immunosuppressive
330 cytokines, such as TGF- β and IL-10.³³ Th17 cells produce pro-inflammatory cytokines
331 such as IL-17, TNF- α , and IL-6, and induce inflammation in the pathogenesis of
332 autoimmune diseases.³⁴ Our results suggest that FAR suppresses the Th2-dominated
333 allergic response and Th17-mediated inflammation via enhancement of Treg induction.
334 These findings imply that FAR would be a good candidate for development of immune
335 tolerance therapies with wide applicability.

336 In conclusion, the present study demonstrated that FAR is a potent Treg
337 cell-inducing agent and shows a suppressive effect against the OVA-induced allergic
338 response and DSS-induced colon inflammation. These findings might contribute to the

339 development of immunomodulatory agents to manage allergy symptoms and IBD.

340

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345

346 **Conflict of interest**

347 The authors declare no conflicts of interest.

348

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446 **Figure captions**

447 **Figure 1. Effects of phytochemicals (A) and glycosylated ferulic acid (B) on the**
448 **TGF- β 1 secretion level in THP-1-derived dendritic cells.** Data are expressed as the
449 means $\pm$ SDs. * $p < 0.05$: significantly different from the control (A) and ferulic acid
450 (FA) treatment (B). FAG, FA glucoside; FAR, FA rutinoid.

451

452 **Figure 2. Effects of FA and FAR on the anaphylactic score and levels of serum**
453 **histamine, total IgE, ovalbumin (OVA)-specific IgE, and total IgA in**
454 **OVA-sensitized mice.** Schematic representation of the OVA-sensitized mouse model
455 (A). Systemic anaphylactic scores (B), and serum levels of histamine (C), total IgE (D),
456 OVA-specific IgE (E), and total IgA (F). Data are expressed as the means $\pm$ SDs. * $p <$
457 0.05 vs. the OVA-sensitized group.

458

459 **Figure 3. Effects of FA and FAR on the population of regulatory T cells in the**
460 **spleen cells of OVA-sensitized mice and on TGF- β 1 secretion from CD11c⁺ cells of**
461 **spleen cells.** Histograms of flow cytometric analysis (A), Treg cell population (B), and
462 TGF- β 1 levels (C). Data are expressed as the means $\pm$ SDs. * $p < 0.05$ vs. the
463 OVA-sensitized group.

464

465 **Figure 4. Effects of FA and FAR on body weight, colon length, clinical score, and**
466 **histology in dextran sulfate sodium (DSS)-treated mice.** Schematic representation of
467 the DSS-treated mouse model (A). Body weight from day 14 to 22 (B), colon length
468 (C), clinical score (D), and histology (E). Data are expressed as the means $\pm$ SDs. * $p <$
469 0.05 vs. the DSS-treated group.

470

471 **Figure 5. Effects of FA and FAR on the population of Treg cells in the spleen cells**
472 **of DSS-treated mice and on cytokine expression in the colon tissues.** Histograms of
473 flow cytometric analysis (A), Treg cell population (B), IL-17 level (C), and TNF- α
474 level (D). Data are expressed as the means $\pm$ SDs. * $p < 0.05$ vs. the DSS-treated group.
475

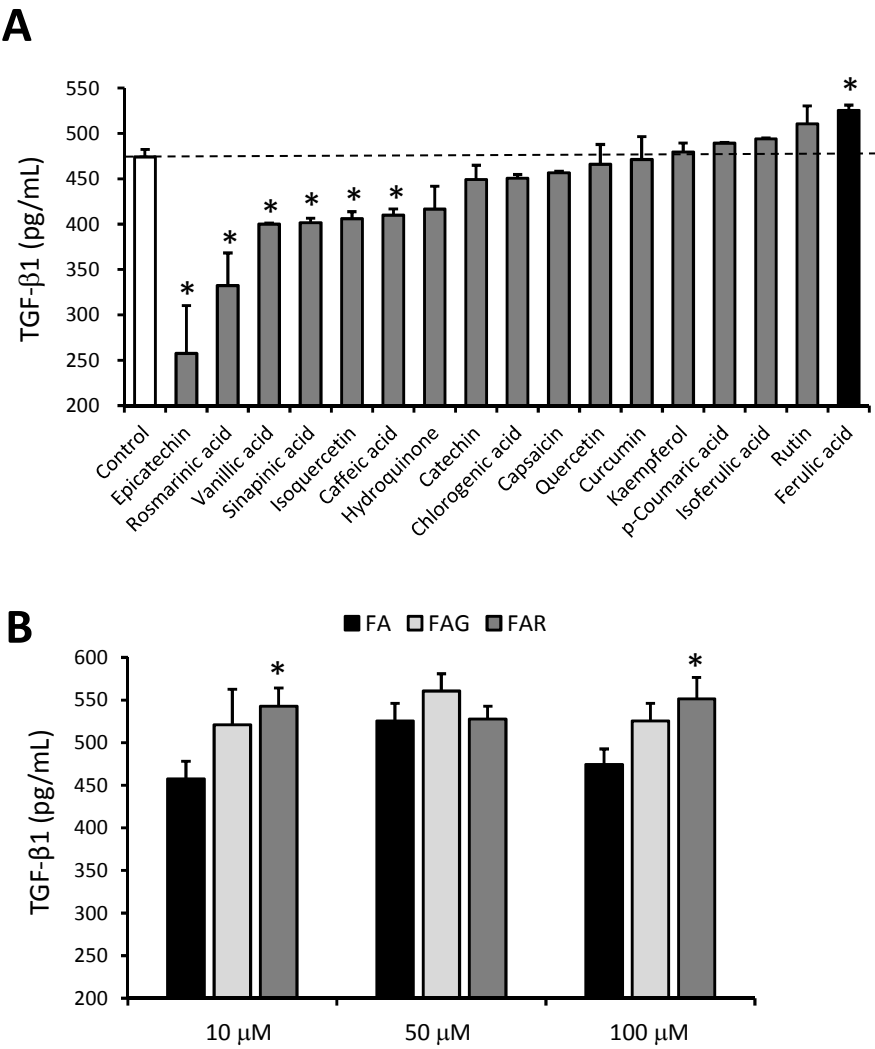


Figure 1
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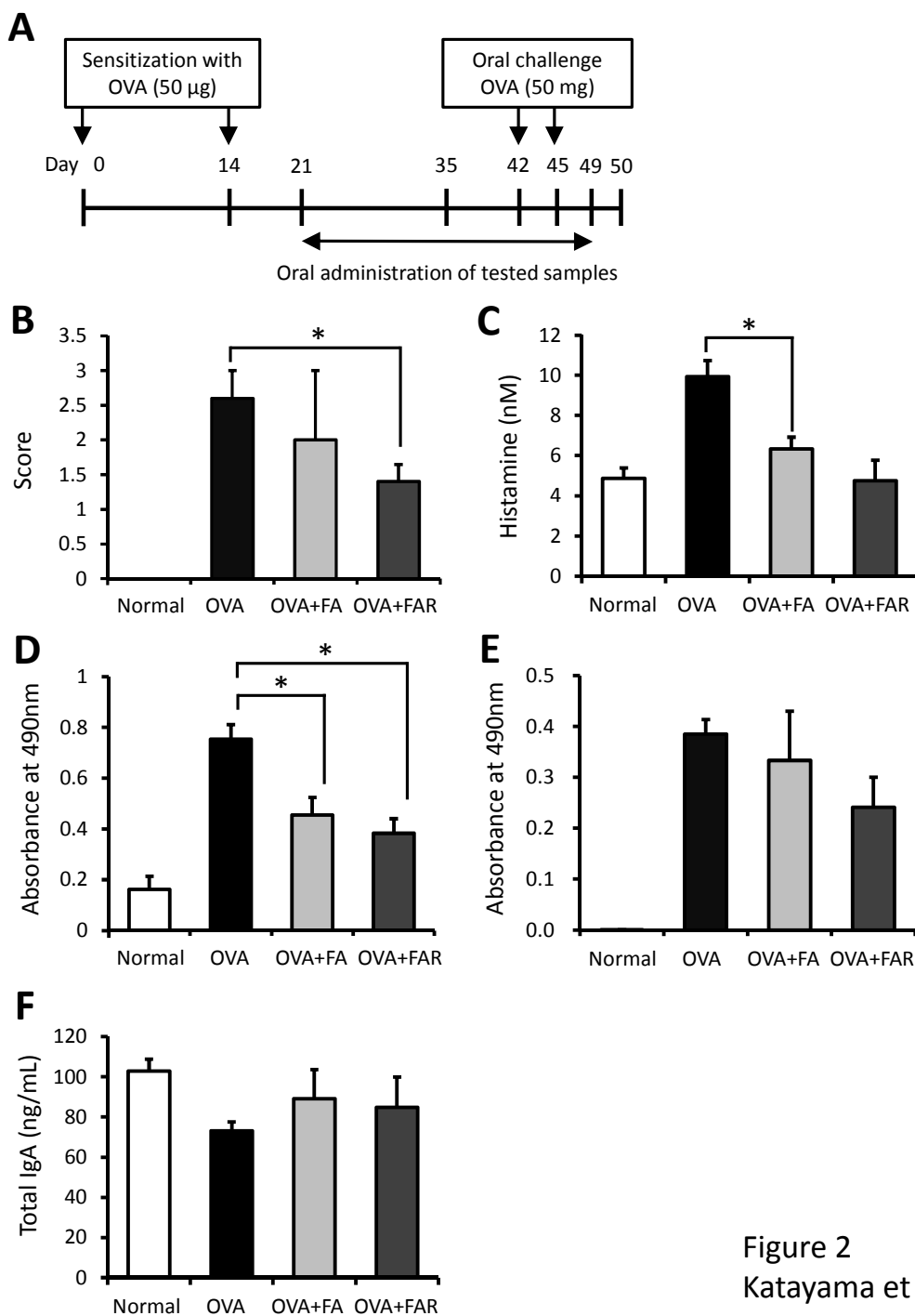


Figure 2
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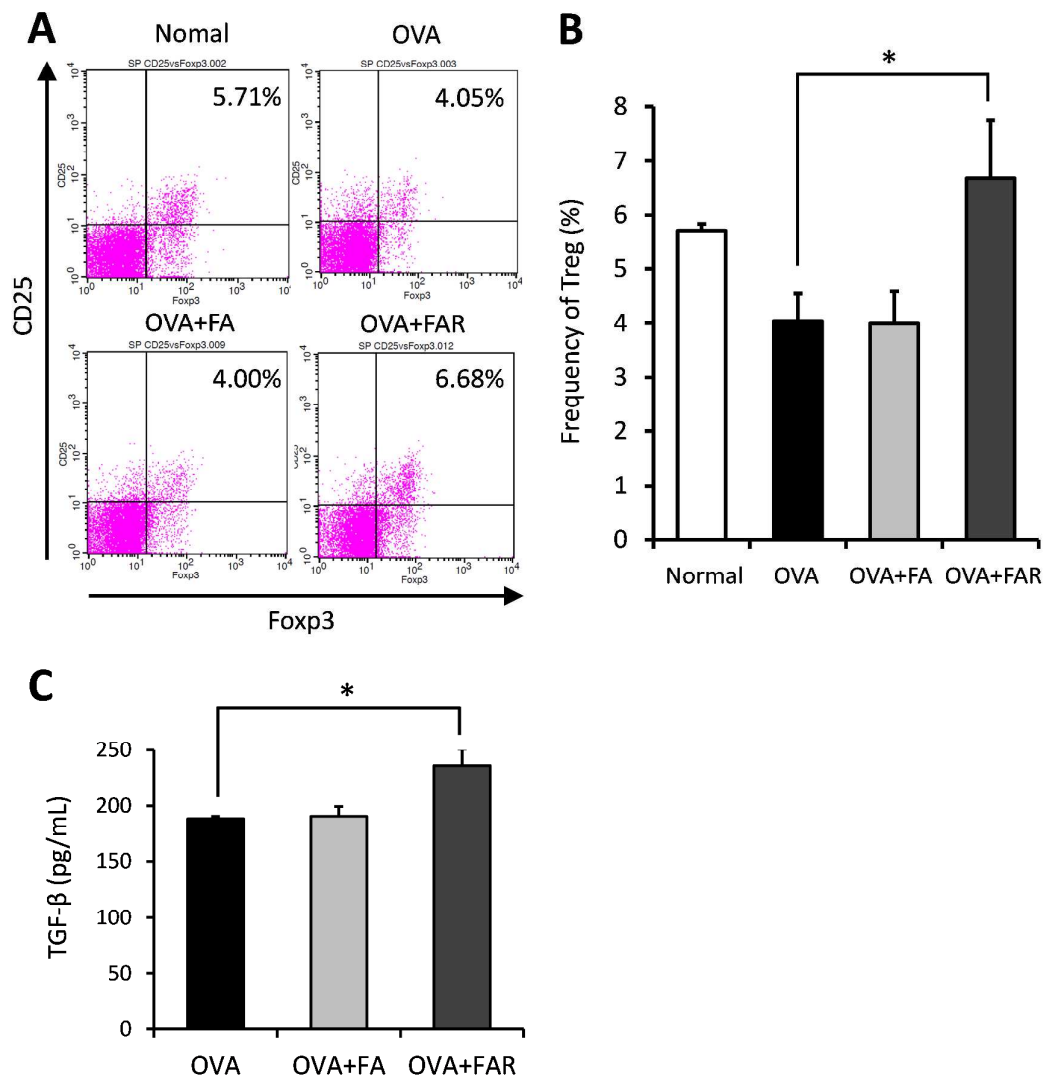
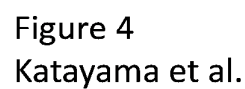


Figure 3
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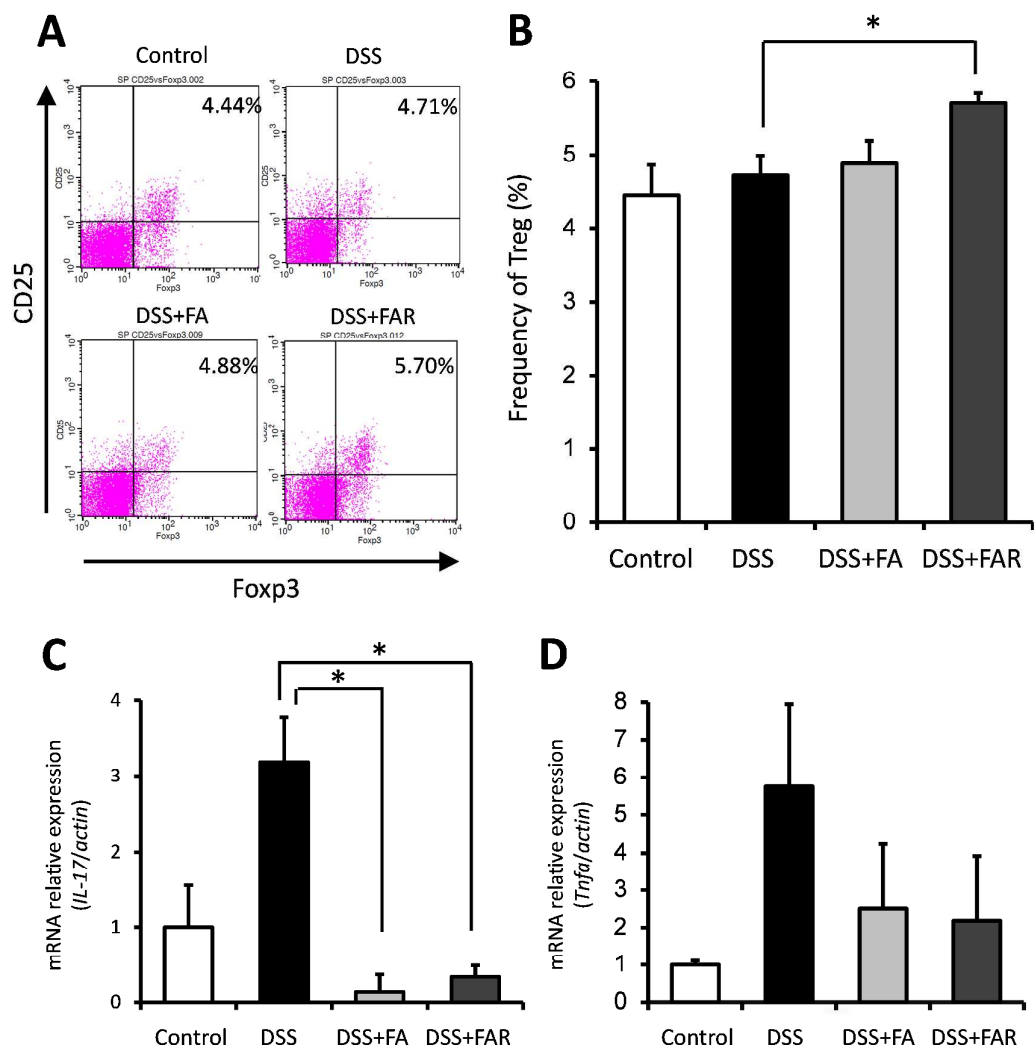
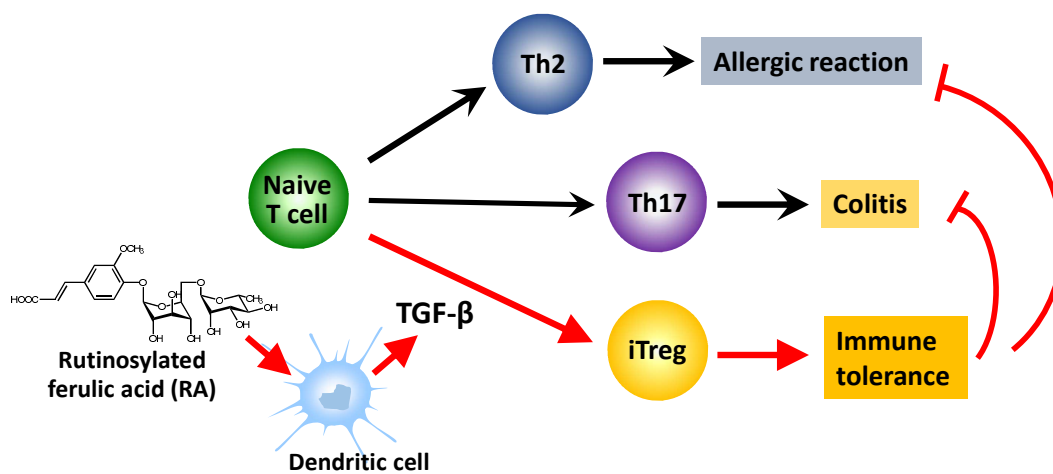


Figure 5
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TOC Graphic
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