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Lycopene intake induces colonic regulatory T cells in mice and suppresses food allergy symptoms

Short running title: Lycopene induces Treg and suppresses food allergy

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Abstract

Background Food allergy (FA) is a common disease in children; thus, a high level of safety is required for its prevention and treatment. Colonic regulatory T cells (Tregs) have been suggested to attenuate FA. We investigated the Treg-inducing ability and anti-FA effects of carotenoids, a pigment contained in vegetables and fruits.

Methods C57BL/6N mice were fed a diet containing 0.01% (w/w) of lycopene, β-carotene, astaxanthin, or lutein for 4 weeks, and the population of colonic Tregs was assessed. Subsequently, to evaluate the Treg-inducing ability of lycopene, splenic naïve $CD4^+$ T cells from BALB/c mice were cultured with anti-CD3/CD28 antibody, TGF-β, and lycopene, and the frequencies of Tregs were examined. The effect of 0.1% (w/w) lycopene containing diet on FA was investigated in OVA-induced FA model BALB/c mice.

Results In screening, only lycopene significantly increased the frequency and number of colonic Tregs. Lycopene also increased Treg differentiation in splenic naïve $CD4^+$ T cells. In FA mice, lycopene-feeding significantly increased the number of colonic Tregs and attenuated allergic symptoms. The expression levels of IL4, IL-9, and IL-13 mRNA in colonic mucosa were also significantly reduced by lycopene. IL-9 is known to induce proliferation of mast cells, and we found that lycopene-feeding significantly reduced the number of mast cells in the colonic mucosa of FA mice.

Conclusion Our results suggest that lycopene, a carotenoid present in many common foods on the market, may have the potential to induce colonic Tregs and suppress FA symptoms.

Key message

Lycopene feeding increased colonic Treg, and attenuated allergy symptoms in food allergy mice. In addition, lycopene feeding suppressed IL-9 expression and decreased the number of mast cells

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in colonic mucosa of food allergy mice.

Key words; food allergy, lycopene, regulatory T cell, mast cell, Th9

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Introduction

Food allergy is common in children, and thus its prevention and treatment also require high levels of safety. It has been suggested that regulatory T cells (Treg) in peripheral blood mononuclear cells are decreased in food allergic children[1 2 3]. It is also suggested that Tregs contribute to naturally acquired allergic tolerance in children with food allergies[4 5]. In mice, it is reported that Treg induction in the colonic lamina propria is involved in the attenuation of food allergy[6]. Therefore, it is speculated that colonic Tregs play an important role in suppressing food allergies, and if a food ingredient that increases colonic Treg can be found, it is expected to lead to the establishment of safe and effective preventive and therapeutic methods.

An epidemiological study suggested that healthy children consume more vegetables and fruits after weaning than food allergic children [7]. In particular, it has been found that carotenoids, which are pigments contained in vegetables and fruits, have an anti-allergic effect via an inhibition of specific T cell activation or a reduction of Th2 chemokines [8 9]. Carotenoids are expected to have a Treg-inducing potential, however, details on the matter have not been investigated.

In this study, we screened for Treg-increasing carotenoids in mice. We used lycopene and β-carotene as from carotenes, and lutein and astaxanthin from xanthophylls for screening. The Treg-inducing ability of lycopene was confirmed *in vitro* in naive T cells. The efficacy and mechanism of lycopene on food allergies was evaluated in OVA-induced food allergy model mice.

Methods

Animals and diets

Five-week-old male C57BL/6N mice and five-week-old female BALB/c mice were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). Mice were kept at 18-24°C and 40-70%

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relative humidity, with a 12 h light/dark cycle. They were acclimatized for 1 week before the experiments. Experimental procedures were conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals. All experimental protocols were approved by the Animal Care Committee of Kyoto Prefectural University of Medicine (Permission Number: M29-558).

For screening of carotenoids, mice were fed a standard chow diet (CE-2; CLEA Japan, Tokyo, Japana) containing 0.1% (w/w) soybean oil (FUJIFILM Wako Pure Chemical Co., Osaka, Japan) alone or with 0.01% (w/w) of lycopene, β-carotene, astaxanthin (all from FUJIFILM Wako Pure Chemical Co.), or lutein (ChromaDex Inc., CA, USA), respectively for 28 days.

For the *in vitro* Treg induction study, BALB/c mice were sacrificed by cervical dislocation, and the spleen was collected immediately.

To assess the anti-food allergy effect of lycopene, BALB/c mice were fed a standard chow diet with 0.1% (w/w) soybean oil alone or with 0.1% (w/w) lycopene (KAGOME CO., LTD., Nagoya, Japan).

Isolation of colonic lamina propria cells

Lamina propria mononuclear cells (LPMCs) were isolated from freshly obtained colonic specimens using a Lamina Propria Dissociation Kit (Miltenyi Biotech, Bergisch Gladbach, Germany), as previously described [10].

In vitro Treg differentiation assay

Naïve $CD4^+$ T cells were isolated from the spleens using a mouse naïve $CD4^+$ T cell isolation kit (Miltenyi Biotec K.K.), according to the manufacturer's instructions. The isolated naïve $CD4^+$ T cells were cultured in 96-well round-bottom plates in RPMI 1640 medium containing 10%

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heat-inactivated foetal bovine serum, 1% penicillin–streptomycin, and 59 μM 2-mercaptoethanol. Treg cells were differentiated with 0.25 μL/well Dynabeads Mouse T-activator CD3/CD28 (Life Technologies, Waltham, MA, USA) and recombinant human TGF-β1 (5 ng/mL) (PeproTech Inc., Rocky Hill, NJ, USA), and incubation at 37°C, 5% CO_2 for 96 h. To assess the effect of lycopene on Treg induction, lycopene (1-10 nM) or all-trans retinoic acid (ATRA; all from FUJIFILM Wako Pure Chemical Co.) were also simultaneously added to the culture. The cells were collected, washed, and used in the flow cytometry assays.

Flow cytometry analysis

Cells were permeabilized with FOXP3/Transcription Factor Staining Buffer (eBioscience, Inc., San Diego, USA), and stained with FITC-TCR-beta (BD Biosciences, Bedford, MA, USA), APC-CD25 (BD Biosciences), eFlour780-Fixable Viability Dye (eBioscience), eFlour450-CD4 (eBioscience), V500-CD45 (BD Biosciences), and PE-FOXP3 (eBioscience) under the presence of Fcγ II/III receptor. Cells were analysed using a BD FACS Canto™ II Flow Cytometer instrument with BD FACSDIVA™ and FLOWJO software (version 7.6; Tree Star Inc., San Carlos, CA, USA).

Food allergy sensitization and challenge protocol

Food allergy was induced in BALB/c mice as previously described [11] and depicted in Fig 2A. Following the final OVA challenge (Day 43), diarrhoea score[6] and anaphylaxis score[11] were evaluated as previously described. In addition, rectal temperature was measured using a monitoring thermometer (Natsume Seisakusho Co., Tokyo, Japan). The mice were sacrificed (Day 44), and their colons were removed for macroscopic and histological examination. Colonic specimens were also used for biochemical assays and RNA isolation. In addition, to evaluate

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blood biochemical analysis, plasma was collected by centrifugation and stored at -80°C until further use.

Measurement of immunoglobulin (IgE/IgG1/IgG2a) and mMCP-1 levels in mouse plasma

OVA-specific IgE, IgG1 and IgG2a antibody levels in plasma were determined using ELISA kits (DS Pharma Biomedical Co., Ltd., Osaka, Japan; Chondrex, Inc., Redmond, WA, USA). Mouse mast cell protease 1 (mMCP-1) were also measured using an mMCP-1 ELISA kit (Thermo Fisher, Vienna, Austria).

Analysis of mRNA expression on colonic mucosa

Total RNA was isolated from colonic mucosa using the acid guanidinium phenol chloroform method, using Isogen (Nippon Gene, Co., Ltd., Tokyo, Japan). The resultant cDNA was subjected to quantitative real-time PCR (qRT-PCR) as previously described[12]. Primers were determined by a previously reported method [12] (Table 1).

Immunohistochemistry staining

Formalin-fixed and paraffin-embedded tissue blocks were sectionedand mounted onto MAS-coated slides (Matsunami, Osaka, Japan). After autoclaving for 15 min at 121°C, the sections were incubated at an ambient room temperature of 25°C for 3 h with anti-mouse/rat FOXP3 antibody (eBioscience), which was diluted 1:200 in antibody dilution (Dako). Negative controls for non-specific binding were also processed and revealed no signal. All sections were counterstained with haematoxylin.

Staining of mast cells was carried out in the same way as described above, but with some variations. Specifically, we performed antigen retrieval for 20 min at 95 °C in a microwave oven.

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The sections were incubated at 4°C for 16 h with rabbit monoclonal anti-mast cell tryptase antibody (Abcam, Plc., Cambridge, UK) diluted 1:200 in antibody dilution (TOYOBO Can Get Signal) [13, 14] .

Statistical analyses

Data are presented as mean ± SEM. Statistical significance of differences between groups was determined using a two-tailed unpaired Student's *t*-test (two groups). Alternatively, differences between groups were determined by one-way ANOVA (≥ three groups). To determine differences between individual groups, Tukey's or Dunnett's multiple comparison tests were performed when one-way ANOVAs indicated significant differences. A value of $p < 0.05$ was considered statistically significant. Statistical significance was calculated using GRAPHPAD PRISM (version 6.0; GraphPad Software, Inc., San Diego, CA, USA).

Results

Lycopene increases colonic Treg

The ratio of $FOXP3^+CD25^+$ cells in $CD4^+TCR\beta^+$ lamina propria lymphocytes (LPL) was significantly higher only in lycopene-fed mice (Figure 1A). The numbers of $FOXP3^+$ cells in LPL were also significantly increased in the lycopene-fed mice (Figure 1B-C). The Treg induction ability of lycopene was also shown in *in vitro* Treg differentiation assay (Figure 1 D).

Effect of lycopene on OVA-induced food allergy

The study groups and schedule for food allergy experiments are described in Figure 2A. The number of colonic $FOXP3^+$ cells was significantly increased in the lycopene-fed group (Figure 2B). Lycopene feeding also increased the ratio of $Foxp3^+CD25^+$ cells in LPL (Figure 2C).

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Lycopene feeding significantly suppressed allergic and diarrhoea score (Figure 2E, F). The lycopene-fed group had an ameliorated decrease in rectal temperature, but the difference was not significant.

Lycopene suppresses the expression of colonic Th2 and Th9 cytokines

In the experiments shown in Figure 2, lycopene feeding significantly suppressed the expression of Th2-related (*Il-4* and *Il-13*) and Th9-related (*IL-9*) cytokines (Figure 3A, C, D). There was no change in *Il-5* production due to lycopene intake (Figure 3B).

Lycopene does not improve the OVA-specific immunoglobulin and the expression of tight junction mRNAs in the colonic mucosa

In the experiments shown in Figure 2, the concentrations of Th2-related Igs (IgE and IgG1) and Th1-related Ig (IgG2a) [15] in plasma were measured. The concentration of OVA-specific IgG1 was significantly decreased with lycopene feeding, but those of OVA-specific IgE and IgG2a were not significantly affected (Figure 4A-C).

IL-13 has been reported to disrupt intestinal tight junctions [16 17]. However, lycopene feeding showed no effect on the expression of tight junction mRNAs (Figure 4D-K).

Lycopene suppresses Th9-related colonic mast cell proliferation

IL-9 has been reported to increase in intestinal mast cells in food allergies [18]. In the experiment shown in Figure 2, the number of mast cell tryptase$^+$ cells were significantly decreased in the lycopene-fed group (Figure 5A, B). The plasma level of mMCPt-1, a marker of mast cell degranulation [19], was significantly suppressed in lycopene-fed group (Figure 5C).

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Discussion

In this study, the Treg-inducing ability and the suppressive effect on food allergy of carotenoids was evaluated in a mouse model. As a result, lycopene feeding increased colonic Treg count and improved allergy symptoms.

In this study, lycopene promoted Treg differentiation in an *in vitro* assay. Thus, it was suggested that lycopene directly regulates the differentiation of Tregs. Similar to lycopene, retinoic acid also induces Treg differentiation by direct action on naïve $CD4^+$ T cells. Orally ingested lycopene is reported to be metabolised and activates the retinoic acid receptor [20] [21]. Therefore, it is speculated that one of the Treg-inducing pathways of lycopene *in vivo* may be mediated by the activation of retinoic acid receptors.

On the other hand, in this study, Treg induction by intact lycopene was also observed *in vitro*. Intact lycopene weakly activates the retinoic acid receptor [22]. Therefore, it is thought that Treg induction by lycopene has a pathway other than the activation of retinoic acid receptors. It has been reported that Tregs are efficiently induced by the weak stimulation of the Akt/mTOR signal pathway by T-cell receptor (TCR) stimulation [23]. It has also been reported that Treg induction is enhanced by adding rapamycin, an mTOR inhibitor, to an *in vitro* assay that activates T cells with the anti-TCR/CD28 antibody [24]. Previously, it was shown that the mTOR protein was decreased in the liver of lycopene-fed mice[25]. Inhibition of mTOR is considered to be exerted by intact lycopene because the effect of lycopene was confirmed in knockout mice of beta-carotene-90,100-oxygenase, a lycopene-metabolizing enzyme [25]. Therefore, as one of the Treg-inducing pathways of lycopene *in vitro*, a pathway via the suppression of mTOR is proposed. However, since the previous report used mouse liver cells, further verification is required to determine whether lycopene reduces mTOR expression in T cells. In this study, Treg-inducing carotenoids were screened only at a dietary dose of 0.01% (w/w). Therefore, it should be noted

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that the possibility of exerting Treg-inducing ability with other carotenoids cannot be ruled out at higher doses.

The T cell subsets involved in the development of food allergies include Th2 and Th9, which are regulated by Treg differentiation [26] [27]. Th2 mainly produces IL-4, IL-5, and IL-13. Each cytokine contributes to the development of allergies through allergen-specific IgE production, promotion of eosinophil migration, and an increase in allergen permeability due to the destruction of tight junctions [28]. On the other hand, Th9 contributes to the increase in mast cells, which are the final effector cells of allergic reactions, through the production of IL-9 [18]. In this study, lycopene consistently decreased the mRNA expression of IL-9 and mast cell number in colonic lamina propria. Therefore, it is speculated that the induction of Treg by lycopene may have mainly contributed to the suppression of Th9 differentiation.

IL-9 produced by Th9 cells accumulates mast cells in tissues [29]. Mast cells in the intestinal tissue are degranulated by allergens and induce diarrhoea in food allergy [19]. Chen et al. suggested that diarrhoea and a decrease in body temperature by administration of OVA are related to the number of mast cells, but not the anti-OVA-IgE antibody titre. Therefore, it is speculated that the effect of lycopene feeding on food allergy symptoms was mediated by the Treg-Th9-mast cell pathway. In addition, Chen et al. have shown that IL-9-producing mast cells (MMC9s) particularly contribute to the induction of diarrhoea [30]. Unfortunately, this study did not evaluate MMC9s numbers. However, it has been reported that MMC9s produce IL4 and IL-13 at the same level as Th2, but that they have an extremely low IL-5 producing ability [30]. The changes in cytokine expression shown in Figure 3 are considered consistent with a decrease in MMC9s. The expression of cytokine mRNAs in intestinal tissues the day after OVA challenge has been interpreted as T cell-derived [11, 31], but our experiments could not exclude mast cell-derived mRNAs from the analysis. It has been suggested that MMC9s are also increased in food allergic

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patients [30]. However, since there are no reports of food components that can reduce MMC9s, it is expected that if the inhibitory action of lycopene on MMC9s is confirmed, it can contribute to the establishment of a new preventive or therapeutic strategy in human food allergy patients.

In the future, verification of the effect of lycopene in humans is expected, but the dose of lycopene is an issue. In this study, the lycopene intake of the mouse was estimated as 150 mg/kg body weight/day. Converting this to an effective dose in humans based on body surface area, it is 12 mg/kg body weight/day for adults and 18 mg/kg body weight/day for children. For example, if an adult weighs 60 kg and a child weighs 20 kg, it is necessary to take 1,080 mg/day and 360 mg/day of lycopene, respectively. In commercial tomato juice, the lycopene content is 35 mg/bottle[32]. Thus, we should first confirm the effectiveness of lycopene at the dose of general foods in human intervention studies.

In the present study, we found that lycopene induced colonic Treg and suppressed food allergy symptoms in mice. Lycopene is a carotenoid contained in many common foods on the market such as tomato, watermelon, pink grapefruit, and papaya, and we can include it in our daily diet with ease. Thus, it may be possible to prevent and improve food allergies by improving an individual's diet so that lycopene is actively consumed. In order to prevent and improve food allergies, it is expected that a comprehensive and effective plan is to be developed. A plan that not only relies on medicines but one that also combines dietary factors.

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Conflict of Interest Statement

NF, KA, and HS are employees of KAGOME CO., LTD, which sells products containing lycopene. NF and KA hold stock in KAGOME CO., LTD. NF has a patent pending related to this work. The Kyoto Prefectural University of Medicine received financial support from KAGOME CO., LTD. for this collaborative research.

Authorship

YI and YN directed the paper's conception. CU, TT, and NF wrote the paper. Yasuki H, AH, KK, KU, TI, KA, HS, and YN provided intellectual support and modified the language. CU, KM, Yasuko H, and Yasuki H performed the experiments. All authors have read and approved the final manuscript for publication.

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TABLE 1 Primers used in this study

	Forward	**Reverse**
Actin	TATCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTA
Il4	CGCCATGCACGGAGATG	CGAGCTCACTCTCTGTGGTGTT
Il5	TGACCGCCAAAAAGAGAAGTGT	ACTCTTGCAGGTAATCCAGGAACT
Il13	CGCAAGGCCCCCACTA	AAAGTGGGCTACTTCGATTTTGG
Il9	CTTGCCTCTGTTTTGCTCTTCA	CCCCATGTGGTGCTGCAT
Zo1	CGCCAAATGCGGTTGATC	TTTACACCTTGCTTAGAGTCAGGGTTA
Zo2	TCAAGCCAACAAGCTCAAAAAG	ATCGTTGGCTGAATTCACGTT
JAM	TGCGGACCGAGTCACCTT	CTTCCGGGTCACAGAACTGAA
Occudin	AGCCTCGGTACAGCAGCAAT	CCTTCGTGGGAGCCCTTT
Claudin1	TGACCGCTCAGGCCATCTAC	CTGCCCGGTGCTTTGC
Claudin2	CCTCCCTTGGCGTCCAA	GTGCCTAACAGCCCCAAAAG
Claudin3	TCATCACGGCGCAGATCA	CTCTGCACCACGCAGTTCA
Claudin4	TGTCCTGGACCGCTCACAA	CCCGGAAGCCACCATAGG

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Figure legends

FIGURE 1 Lycopene increases colonic Treg in mice. (A) C57BL/6N mice were fed a diet containing 0.01% (w/w) of lycopene, β-carotene, astaxanthin, or lutein for 4 weeks. After sacrifice, colonic lamina propria cells were collected and stained with anti-CD4 antibody, anti-FOXP3 antibody, and anti-CD25 antibody. The cells were analysed by flow cytometry, and the ratio of $FOXP3^+CD25^+$ cells (Treg) to $CD4^+$ cells was calculated. n = 6, mean + SEM, $*P < 0.05$, one-way ANOVA and Dunnett's multiple comparisons vs. control. (B, C) C57BL/6N mice were fed with a diet containing 0.1% (w/w) of lycopene for 4 weeks. After sacrifice, colon tissue was immunohistochemically stained with anti-FOXP3 antibody, and the number of $FOXP3^+$ cells was counted 3 times per mouse. (B) Representative histology of colon samples. Arrowheads indicate $FOXP3^+$ cells. Bar is 50 μm. (C) The data of (B) are summarized. Each symbol indicates an individual value. The horizontal bar indicates the mean. HPF; High Power Field. n = 20, Student's *t*-test. (D) Spleens were collected from BALB/c mice and homogenized to obtain splenocytes. Then naive $CD4^+$ T cells were isolated and cultured in the presence of anti-CD3/CD28 antibody and TGF-β for 96 hours. Lycopene (1 nM or 10 nM) was added from the start of culture to investigate the effect of lycopene on Treg induction. All trans retinoic acid (ATRA; 1 nM) was added as a positive control. After the cultivation, ratio of $FOXP3^+CD25^+$ cells were analysed by flow cytometry. n = 4, mean + SEM, $*P < 0.05$, $**P < 0.01$, one-way ANOVA and Dunnett's multiple comparisons vs. control.

FIGURE 2 Lycopene feeding induces colonic Treg and suppresses allergic symptoms in OVA-induced food allergy model mice. BALB/c mice were injected with 20 mg ovalbumin (OVA) and 2 mg aluminium hydroxide (Alum) intraperitoneally (i.p.) twice at 2-week intervals. From 2 weeks after last i.p., 50 mg OVA solution was intra-gastric administered (i.g.) 6 times in

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the frequency of once every 3 days to induce food allergic shock. Controls (sham) mice that did not induce food allergies received equal doses of PBS (-) i.p. and i.g. Feeding of the control diet and lycopene diet (0.1% (w/w) lycopene containing) was continued from the start of OVA + Alum administration to the end of the study. (A) Schedule for food allergy induction. (B) After sacrifice, the colon was collected and immunohistochemically stained with anti-FOXP3 antibody. The graph shows the number of $FOXP3^+$ cells in each group. Each symbol shows an individual value, and the horizontal bar shows the mean value (n = 11). (C) Lamina propria cells were prepared from colonic tissue, and then stained with anti-CD4 antibody, anti-TCRβ antibody, anti-FOXP3 antibody, and anti-CD25 antibody. Then, the cells were analysed by flow cytometry, and after extracting the data of $CD4^+TCR\beta^+$ cells by gating, the ratio of $FOXP3^+CD25^+$ cells were calculated. The graph shows the mean + SEM of each group. (D-F) At the end of the study, mice were orally administered OVA to evaluate rectal temperature (D), allergic score (E), and diarrhoea score (F). (D, E) Mean ± SEM, (F) Each symbol indicates an individual value, and the horizontal bar indicates means. n = 10. $*P < 0.05$, $**P < 0.01$ vs. sham group, $\#P < 0.05$, $\#\#P < 0.05$ vs. allergy-control group, Tukey's multiple comparison test.

FIGURE 3 Lycopene feeding suppresses the expression of colonic Th2 and Th9 cytokines in OVA-induced food allergy model mice. In the experiments shown in Figure 2, mRNA expression levels of the Th2 cytokines IL-4 (A), IL-5 (B), IL-13 (C), and the Th9 cytokine IL-9 (D) in the colonic mucosa were evaluated. n = 10, mean ± SEM, $*P < 0.05$, $**P < 0.01$ vs. sham group, $\#P < 0.05$, $\#\#P < 0.05$ vs. allergy-control group, Tukey's multiple comparison test.

FIGURE 4 Lycopene feeding does not improve Th2-related antibody production and tight junction-related gene expression. In the experiments shown in Figure 2, plasma OVA-specific IgE

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(A), IgG1 (B), IgG2a (C) titres, and mRNA expression levels of tight junction-related genes Zo-1 (D), Zo-2 (E), JAM (F), occludin (G), claudin 1 (H), claudin 2 (I), claudin 3 (J) and claudin 4 (K) in colonic mucosa were evaluated. n = 10, mean ± SEM, **P* < 0.05, ***P* < 0.05 vs. sham group, ##*P* < 0.05 vs. allergy-control group, Tukey's multiple comparison test.

FIGURE 5 Lycopene feeding suppresses Th9-related mast cell proliferation. In the experiment shown in Figure 2, the number of mast cell tryptase$^+$ cells in colon tissue (A, B) and plasma mast cell protease-1 (mMCPt-1) concentration (C) were evaluated. (A) Representative histology of colon samples. Arrowheads indicate mast cell tryptase$^+$ cells (B) The results of (A) are summarized. Each symbol shows an individual value, and a horizontal line shows mean. n = 19, mean ± SEM, **P* < 0.001 vs. sham group, #*P* < 0.05, ##*P* < 0.01 vs. allergy-control group, Dunn (B) or Tukey's (C) multiple comparison test.

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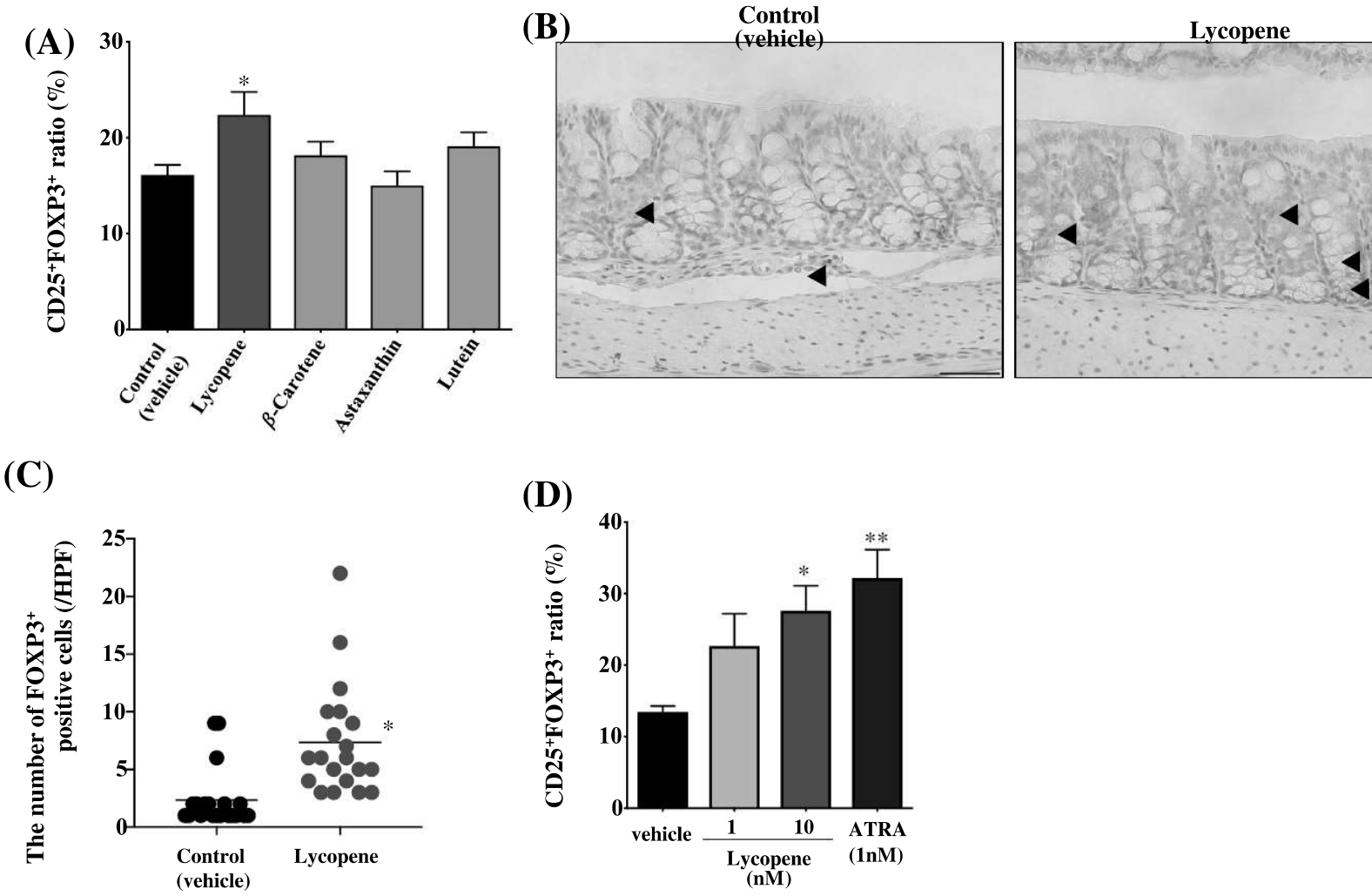
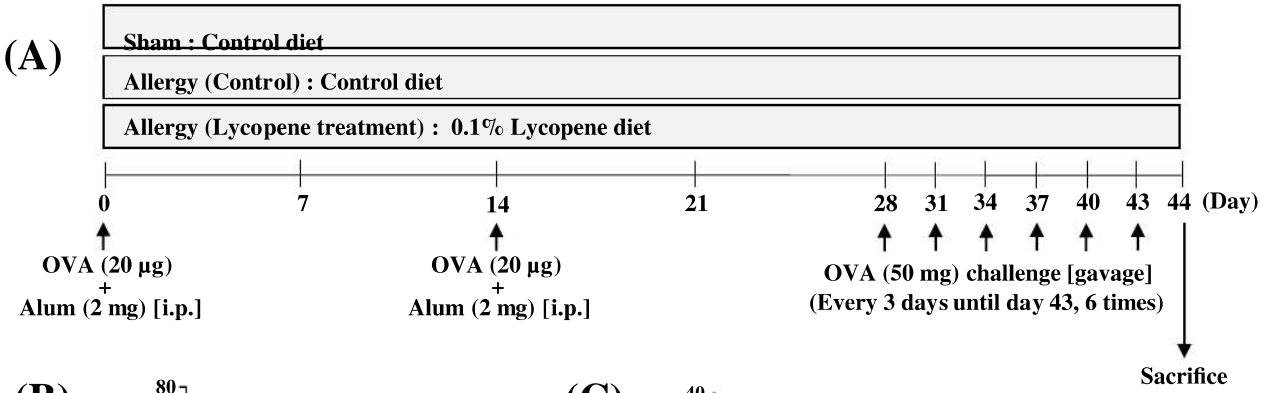
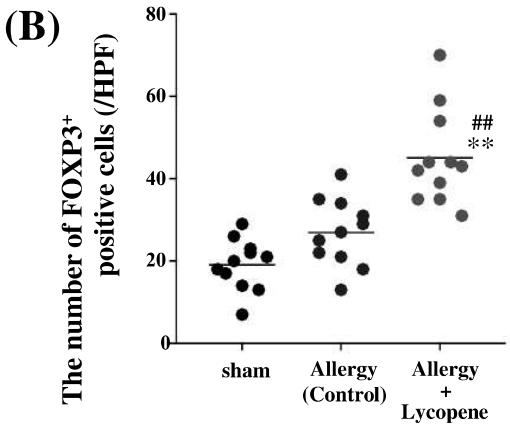
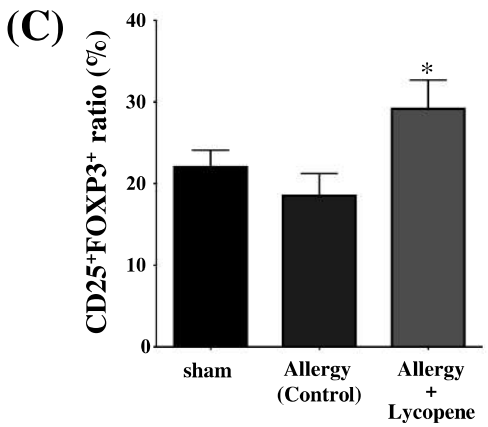
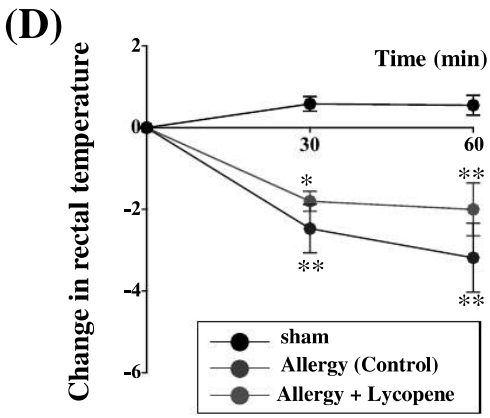

(A)
CD25+FOXP3+ ratio (%)
30
20
10
0
*
Control (vehicle)
Lycopene
β-Carotene
Astaxanthin
Lutein
(B)
Control (vehicle)
Lycopene
(C)
The number of FOXP3+ positive cells (/HPF)
25
20
15
10
5
0
*
Control (vehicle)
Lycopene
(D)
CD25+FOXP3+ ratio (%)
40
30
20
10
0
*
**
vehicle
1
10
Lycopene (nM)
ATRA (1nM)

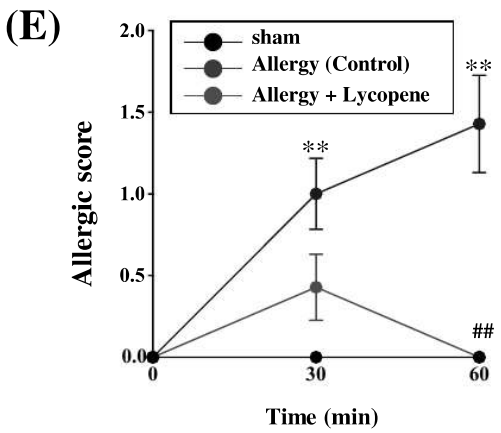
Figure 1

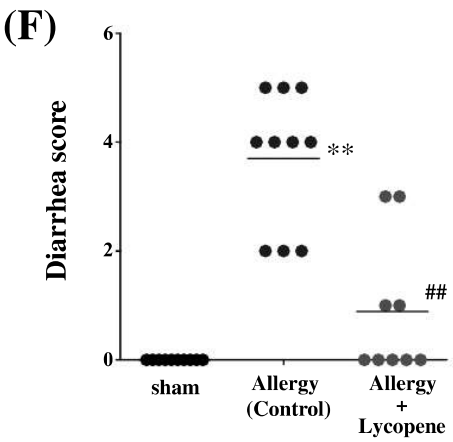

(A)
Sham : Control diet
Allergy (Control) : Control diet
Allergy (Lycopene treatment) : 0.1% Lycopene diet
0
7
14
21
28
31
34
37
40
43
44 (Day)
OVA (20 μg) + Alum (2 mg) [i.p.]
OVA (20 μg) + Alum (2 mg) [i.p.]
OVA (50 mg) challenge [gavage] (Every 3 days until day 43, 6 times)
Sacrifice


(B)
The number of FOXP3+ positive cells (/HPF)
sham
Allergy (Control)
Allergy + Lycopene


(C)
CD25+FOXP3+ ratio (%)
sham
Allergy (Control)
Allergy + Lycopene


(D)
Change in rectal temperature
Time (min)
sham
Allergy (Control)
Allergy + Lycopene


(E)
Allergic score
sham
Allergy (Control)
Allergy + Lycopene
Time (min)


(F)
Diarrhea score
sham
Allergy (Control)
Allergy + Lycopene

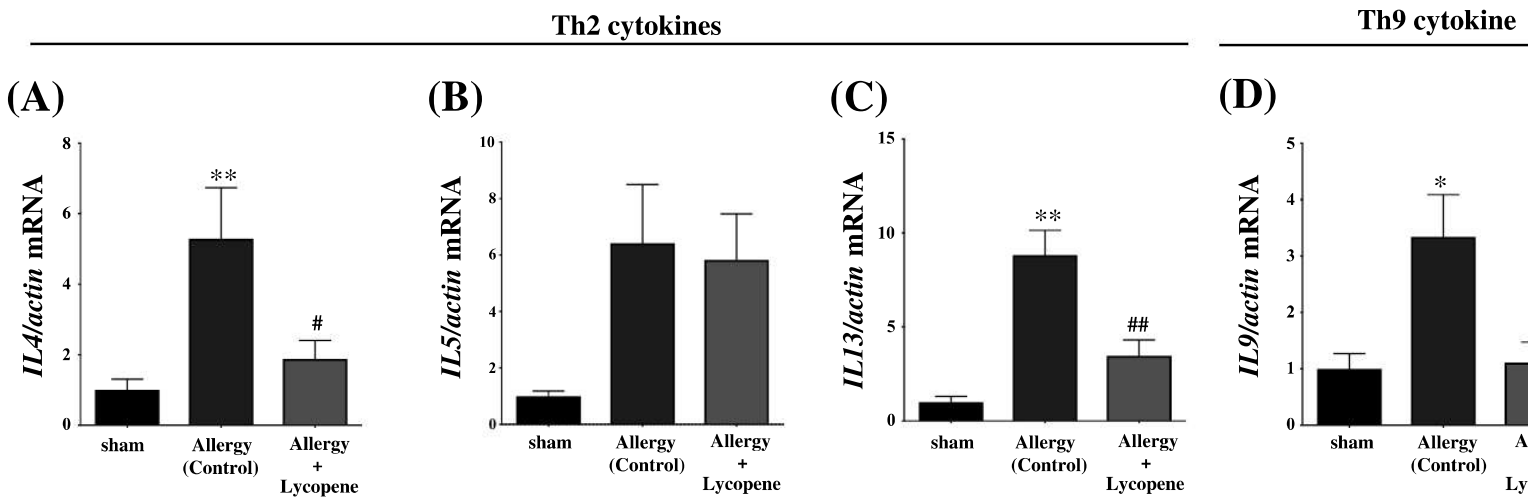

Th2 cytokines
Th9 cytokine
(A)
(B)
(C)
(D)
IL4/actin mRNA
IL5/actin mRNA
IL13/actin mRNA
IL9/actin mRNA
sham
Allergy (Control)
Allergy + Lycopene
**
#
*
##

Figure 3

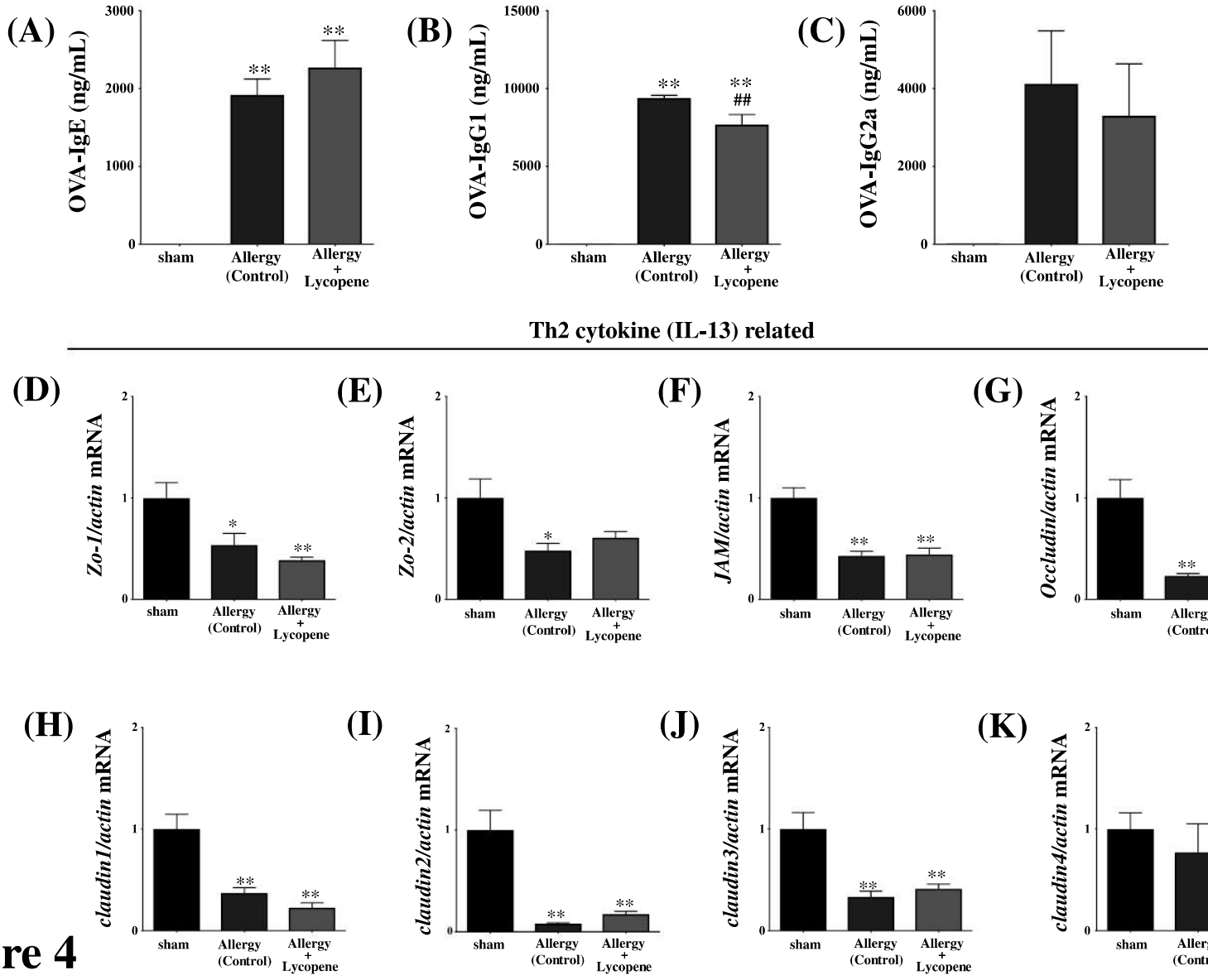

Th2 cytokine (IL-4) related
Th1 cytokine (IFN-γ) related
(A)
OVA-IgE (ng/mL)
3000
2000
1000
0
**
**
sham
Allergy (Control)
Allergy + Lycopene
(B)
OVA-IgG1 (ng/mL)
15000
10000
5000
0
**
**
##
sham
Allergy (Control)
Allergy + Lycopene
(C)
OVA-IgG2a (ng/mL)
6000
4000
2000
0
sham
Allergy (Control)
Allergy + Lycopene
Th2 cytokine (IL-13) related
(D)
Zo-1/actin mRNA
2
1
0
*
**
sham
Allergy (Control)
Allergy + Lycopene
(E)
Zo-2/actin mRNA
2
1
0
*
sham
Allergy (Control)
Allergy + Lycopene
(F)
JAM/actin mRNA
2
1
0
**
**
sham
Allergy (Control)
Allergy + Lycopene
(G)
Occludin/actin mRNA
2
1
0
**
sham
(H)
claudin1/actin mRNA
2
1
0
**
**
sham
Allergy (Control)
Allergy + Lycopene
(I)
claudin2/actin mRNA
2
1
0
**
**
sham
Allergy (Control)
Allergy + Lycopene
(J)
claudin3/actin mRNA
2
1
0
**
**
sham
Allergy (Control)
Allergy + Lycopene
(K)
claudin4/actin mRNA
2
1
0
sham

Figure 4

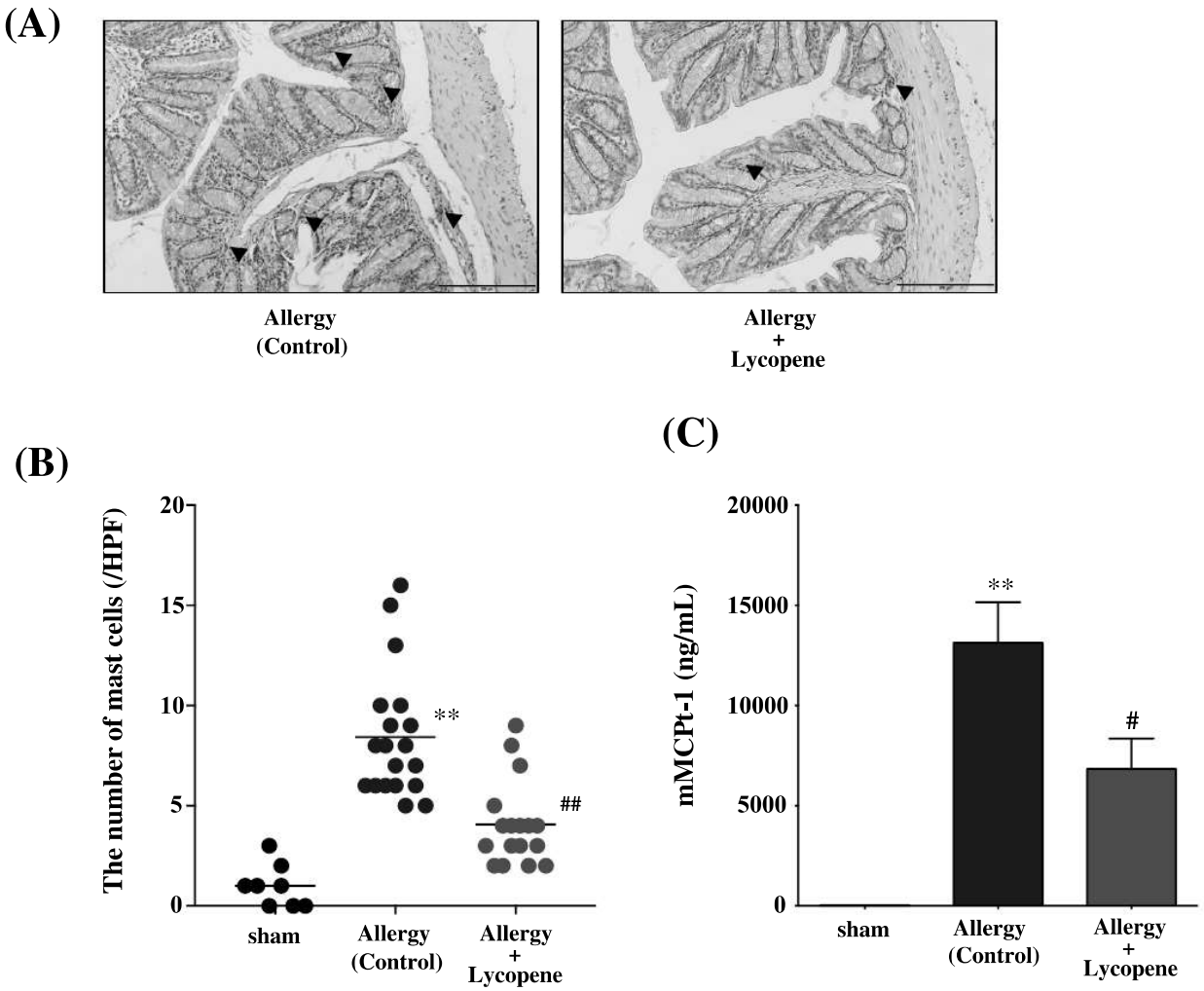

(A)
Allergy
(Control)
Allergy
+
Lycopene
(B)
The number of mast cells (/HPF)
20
15
10
5
0
**
##
sham
Allergy
(Control)
Allergy
+
Lycopene
(C)
mMCPt-1 (ng/mL)
20000
15000
10000
5000
0
**
#
sham
Allergy
(Control)
Allergy
+
Lycopene

Figure 5