Tocotrienol (Unsaturated Vitamin E) Suppresses Degranulation of Mast Cells and Reduces Allergic Dermatitis in Mice

Tsuyoshi Tsuduki¹*, Keiko Kuriyama², Kiyotaka Nakagawa² and Teruo Miyazawa²

¹ Laboratory of Food and Biomolecular Science, Graduate School of Agriculture, Tohoku University (1-1 Tsutsumidori amamiyamachi, Aobaku, Sendai 981-8555, JAPAN)
² Food and Biodynamic Chemistry Laboratory, Graduate School of Agriculture, Tohoku University (1-1 Tsutsumidori amamiyamachi, Aobaku, Sendai 981-8555, JAPAN)

Abstract: In this study, we examined whether tocotrienol (T3) reduces allergic dermatitis in mice and suppresses degranulation of mast cells. First, allergic dermatitis was examined in the atopic dermatitis model NC/Nga mouse. Allergic dermatitis was induced using picryl chloride in mice with and without administration of T3 (1 mg/day/mouse). Increases in scratching behavior, dermal thickening, and the serum histamine level were greatly reduced in mice treated with T3, indicating that T3 reduces allergic dermatitis in vivo. Next, the effect of T3 on degranulation of mast cells was examined, since these cells release bioactive substances such as histamine. T3 significantly suppressed degranulation of mast cells and significantly reduced histamine release. The effect of T3 on protein kinase C (PKC) activity was also measured, since suppression of this activity may be associated with the mechanism underlying the antidegranulation effect of T3. T3 significantly suppressed PKC activity. Therefore, we conclude that T3 suppresses degranulation of mast cells and reduces allergic dermatitis in mice through reduction of PKC activity.

Key words: Allergic dermatitis, degranulation, NC/Nga mouse, RBL-2H3, tocotrienol, vitamin E

1 INTRODUCTION

Vitamin E was originally discovered as a micronutrient essential for reproduction¹. The vitamin E family consists of 4 tocopherols (TOC) and 4 tocotrienols (T3): the α, β, γ, or δ isoforms, in which the chroman ring has different numbers and positions of methyl groups. TOC contains a saturated (phytyl) side chain and T3 has an unsaturated (isoprenoid) tail. TOC is present in foods such as vegetable oils and nuts, whereas T3, a minor plant constituent, is abundant in rice bran, palm oil, and annatto seeds². The major physiological activity of vitamin E is its well-defined antioxidative action³,⁴, with α-Toc having the highest activity. However, T3 is attracting interest due to its superior antioxidative⁵,⁶, antihypercholesterolemic⁷-⁹, anti-cancer¹⁰-¹², and anti-angiogenic activities¹³,¹⁴, which differ somewhat from those of TOC. The greater potential therapeutic effects of T3 compared to those of TOC make the tocotrienols particularly interesting.

The distribution of vitamin E in vivo has been widely studied. A high level of T3 is found in adipose tissue, while...
TOC is widely distributed in the plasma and all organs\textsuperscript{15-17}. We also found that T3 is rapidly transferred to the skin after administration\textsuperscript{18}, which suggests that the skin may be a site for the beneficial effects of T3.

Allergic dermatitis (AD) such as atopic dermatitis is a typical disease of the skin that is classified as a type I allergy\textsuperscript{19}. This type of allergy causes symptoms such as itching, which reduces quality of life. The number of patients with type I allergy is increasing and countermeasures are required. The symptoms of type I allergies are caused by degranulation of mast cells\textsuperscript{20, 21}, which is induced by antigens such as ticks in the skin. Allergy symptoms such as itching are then caused by physiologically active substances such as histamine, which are contained in the granules released from the mast cells. Therefore, suppression of mast cell degranulation should lead to reduction of AD.

In this study, we examined whether T3 reduces AD in mice and suppresses degranulation of mast cells. First, symptoms associated with AD were examined in the atopic dermatitis model NC/Nga mouse. The effect of T3 on mast cell degranulation was then examined in rat basophilic leukemia cells. The results suggest that T3 has efficacy for reduction of AD.

\section*{2 EXPERIMENTAL PROCEDURES}

\subsection*{2.1 Materials}

Rice bran tocotrienol (RBT3) was kindly provided by Sanwa Yushi Co. Ltd. (Tendo, Japan). RBT3 was composed of 97.5\% tocotrienol (T3), 3.5\% \(\alpha\)-T3, 89.9\% \(\gamma\)-T3, and 4.1\% \(\delta\)-T3) and 2.1\% tocopherol (TOC; 1.4\% \(\gamma\)-TOC and 0.7\% \(\delta\)-TOC) (wt/wt). Vitamin E-stripped corn oil was purchased from Acros Organics (Fairlawn, NJ). Picryl chloride (PiCl) was purchased from Tokyo Kasei Chemical Co. Ltd. (Tendo, Japan). PiCl was recrystallized from 100\% ethanol was used to prepare solutions, which were always made just before use and kept shielded from light\textsuperscript{22-24}. \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\)-T3 (purity 98\%) and \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\)-TOC (purity 98\%) were purchased from Chromadex (Santa Ana, CA). WST-1 reagent was purchased from Dojindo Laboratories (Kumamoto, Japan). All other reagents were of analytical grade.

\subsection*{2.2 Methods}

\subsubsection*{2.2.1 Animals and diets}

All procedures were performed in accordance with the Animal Experiment Guidelines of Tohoku University. The animal protocol was approved by the Animal Use Committee at Tohoku University. Male NC/Nga mice (5 weeks of age) were obtained from Japan SLC (Hamamatsu, Japan). After acclimatization to a commercial diet (MF; Oriental Yeast, Tokyo, Japan) for 1 week, the mice were divided into three groups: those not sensitized and given no T3 treatment (control group); those sensitized and challenged to develop AD, but given no T3 treatment (AD group); and those fed RBT3 at 1 mg/day/mouse and sensitized and challenged (AD + T3 group). The mice were housed 6 per cage with free access to commercial diets and distilled water in a temperature- and humidity-controlled room with a light cycle of 12 h on and 12 h off\textsuperscript{25, 26}. Each mouse received T3 (dissolved in 50\,\mu\text{L} of vitamin E-stripped corn oil) or vitamin E-stripped corn oil alone orally once a day. To examine the protective efficacy of T3, only the operation of T3 administration was done to mice for one week. After one week of the start of T3 administration, sensitization and challenge were performed as previously described\textsuperscript{22-24}. In brief, fur of the thoracic and abdominal regions was shaved off with a hair clipper under anesthesia 1 day before sensitization. Using a micropipette, 150\,\mu\text{L} of sensitizing 5\% PiCl in ethanol/acetone (4:1 mixture) was applied to the thoracic and abdominal areas, as well as to the soles of the hind paws. The fur of the back region was shaved off with a hair clipper under anesthesia 1 day before challenge. Challenge was performed 4 days after sensitization. A micropipette was used to apply 150\,\mu\text{L} of 1\% PiCl in corn oil (dissolution was achieved by heating) to the back and ears. The procedure was repeated once a week for up to 9 weeks. Then, scratching behavior was observed, the mice were anesthetized by diethyl ether and sacrificed by decapitation, and the skin and serum were collected and stored at \(-80^\circ\text{C}\) until performance of assays. Pieces of dorsal skin were fixed in 10\% formalin.

\subsubsection*{2.2.2 Scratching behavior}

Scratching behavior was observed as described previously\textsuperscript{24, 27}. Before behavioral recording, the mice were put into an acrylic box composed of four cells for at least 1 h for acclimation. Thereafter, their behavior was videotaped for 20 min without any research staff in the observation room. Playback of the video was used to count scratching events. Scratching of any region of the body by the hind paws was counted as spontaneous scratching. Mice rapidly scratched several times for about 1 s and a series of these movements was counted as one scratching event.

\subsubsection*{2.2.3 Skin histology analysis}

To examine thickening of the epidermis, mouse skin was fixed in 10\% formalin and embedded in paraffin\textsuperscript{24, 25}. Vertical sections (5\,\mu\text{m}) were cut, mounted on a glass slide, stained with hematoxylin and eosin, and observed using a microscope (BZ-8000; Keyence, Osaka, Japan).

\subsubsection*{2.2.4 Biochemical analyses in serum and culture medium}

Histamine levels in serum and culture medium were determined using a histamine EIA kit (Spi-bio, Montigny-le-Bretonneux, France). IgE levels in serum were determined using ELISA kits (Shibayagi, Shibukawa, Japan)\textsuperscript{27}. Measurement of T3 and TOC concentrations in the skin

T3 and TOC in the skin and cultured cells were extracted as described previously\textsuperscript{18, 28}. Concentrations of T3 and TOC...
were determined by fluorescence HPLC (FL-HPLC). Separation was performed at 35°C using a silica column (ZORBAX Rx-SIL, 4.6 x 250 mm; Agilent, Palo Alto, CA). A hexane/1,4-dioxane/2-propanol (98:8:10) mixture was used as the mobile phase at a flow rate of 1.0 mL/min. T3 and TOC isoforms were detected and determined using an RF-10AXL FLD detector (excitation 294 nm, emission 326 nm; Shimadzu, Kyoto, Japan).

2.2.6 Cells and Cell Culture

Rat basophilic leukemia (RBL-2H3) cells were obtained from the Cell Resource Center for Biomedical Research at Tohoku University School of Medicine (Sendai, Japan). RBL-2H3 cells were cultured in a growth medium (Dulbecco’s modified Eagle’s medium, DMEM; Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Dainippon Pharmaceutical, Osaka, Japan), 100 kU/L penicillin, and streptomycin (100 mg/L) (Gibco BRL Rockville, MD) at 37°C in a 5% CO2/95% air atmosphere in a humidified incubator.

2.2.7 Preparation of Test Medium

T3 or TOC was dissolved in ethanol at 50 mM concentration. The solution was diluted with test medium (DMEM containing 0.5% FBS) to achieve the desired final concentration (0-50 μM). The final concentration of ethanol in the test medium was up to 0.1% (v/v), which did not affect cell viability. Medium containing only vehicle (0.1% ethanol) was prepared and used as a control in the study.

2.2.8 β-Hexosaminidase Release Assay

Inhibitory effects on the release of β-hexosaminidase and histamine in RBL-2H3 cells were evaluated using a reported method. Briefly, RBL-2H3 cells were plated in 96-well tissue culture plates (1 x 10^3 cells/well) and incubated for 24 h. After the cells were washed with PBS, the medium was replaced with 1 μg/mL DNP-IgE in DMEM (100 μL/well). After incubation for 12 h, the medium was replaced with the test medium (100 μL/well). After incubation for a further 12 h, the number of viable cells was determined using the water-soluble tetrazolium salt (WST-1) assay. WST-1 is a tetrazolium salt that is converted into a soluble formazan salt by succinate-tetrazolium reductase in the respiratory chain of active mitochondria of viable cells. Briefly, 10 μL of WST-1 solution was added to each well and incubated at 37°C for 3 h and the absorbance (450/655 nm) of the culture medium was measured using a microplate reader (Infinite F200, Tecan, Japan).

2.2.9 Histamine Release Assay

RBL-2H3 cells were plated in 96-well tissue culture plates (1 x 10^3 cells/well) and incubated for 24 h. After the cells were washed with PBS, the medium was replaced with 1 μg/mL DNP-IgE in DMEM (100 μL/well). After incubation for 12 h, the medium was replaced with the test medium (100 μL/well). After incubation for a further 12 h, the cells were washed twice in Tyrode’s buffer and stimulation was initiated by addition of 1 μg/mL DNP-BSA in Tyrode’s buffer (150 μL/well), followed by incubation for 90 min at 37°C. Histamine levels in the supernatant were quantified using a histamine EIA kit, as described above. Spontaneous release of histamine and total histamine were determined for cells in Tyrode’s buffer without antigen or with 0.1% Triton-X-100.

2.2.10 Cell Viability Assay

RBL-2H3 cells were plated in 96-well tissue culture plates (1 x 10^3 cells/well) and incubated for 24 h. After the cells were washed with PBS, the medium was replaced with 1 μg/mL DNP-IgE in DMEM (100 μL/well). After incubation for 12 h, the medium was replaced with the test medium (100 μL/well). After incubation for a further 12 h, the number of viable cells was determined using the water-soluble tetrazolium salt (WST-1) assay.

2.2.11 Protein Kinase C (PKC) Activity

RBL-2H3 cells were preincubated for 24 h. After the cells were washed with PBS, the medium was replaced with 1 μg/mL DNP-IgE in DMEM. After incubation for 12 h, the medium was replaced with the test medium. After incubation for a further 12 h, the cells were washed twice in Tyrode’s buffer and stimulation was initiated by addition of 1 μg/mL DNP-BSA in Tyrode’s buffer, followed by incubation for 90 min at 37°C. Cellular PKC activity was determined using a PepTag non-radioactive PKC assay kit (Promega, Madison, WI, USA).

2.2.12 Measurement of T3 Concentrations in Cultured Cells

RBL-2H3 cells were preincubated in DMEM for 24 h. After the cells were washed with PBS, the medium was replaced with 1 μg/mL DNP-IgE in DMEM. After incubation for 12 h, the medium was replaced with the test medium. After incubation for a further 12 h, the cells were washed twice in PBS. T3 concentrations in RBL-2H3 cells were quantified as described above.

2.2.13 Statistical Analysis

Results are expressed as means ± SE (in the animal study) or means ± SD (in the cell culture study). Data were analyzed by one-way ANOVA, followed by a Tukey honest
significant difference test. A difference was considered to be significant at \( P<0.05 \).

3 RESULTS

3.1 Effect of T3 on atopic dermatitis in NC/Nga mice

The effects of T3 on increases in scratching behavior, dermal thickening, and serum histamine and IgE levels, which are features of AD, were examined in the atopic dermatitis model NC/Nga mouse. AD was induced with PiCl in mice that did (AD + T3 group) or did not (AD group) receive RBT3. Controls did not undergo PiCl sensitization. Scratching behavior in the AD group was significantly higher than that in the control group (Fig. 2A) and was significantly decreased in the AD + T3 group to 50% of that in the AD group. Hematoxylin & eosin staining showed that the epidermis in the AD group was much thicker than that in the control group (Fig. 2B) and that dermal thickening in the AD + T3 group was reduced compared with that in the AD group. The serum histamine level in the AD group was significantly higher than that in the control group (Fig. 2C) and was significantly decreased in the AD + T3 group to 45% of that in the AD group. The serum IgE level in the AD group was also significantly higher than that in the control group (Fig. 2D), but there was no significant difference in the serum IgE level between the AD and AD + T3 groups. Overall, these results show that T3 reduced AD in vivo.

3.2 Vitamin E concentrations in skin of NC/Nga mice fed tocotrienol

T3 concentrations in the skin of mice in the control, AD and AD + T3 groups increased after T3 administration (Table 1), with significant increases in the levels of \( \alpha \)-T3 and \( \gamma \)-T3. In contrast, there was no significant change in the TOC level in each group. These results show that exogenous T3 can reach the skin of mice.

3.3 Effect of T3 on degranulation in RBL-2H3 cells

T3 suppressed the serum histamine level and scratching behavior, but did not influence the IgE level. This indicates that T3 acts downstream from production of IgE, which suggests that T3 may suppress degranulation of mast cells as a mechanism underlying its anti-AD effect. Therefore, the effect of T3 on mast cell degranulation was examined in RBL-2H3 cells. T3 significantly suppressed degranulation in a dose-dependent manner (Fig. 3), with \( \delta \)-T3 having the strongest effect and \( \alpha \)-T3 the weakest. The order of blocking of degranulation was \( \delta \)-T3 \( > \beta \)-T3 \( > \gamma \)-T3 \( > \alpha \)-T3. In contrast, TOC did not have an antidegranulation effect. These results show that T3 is effective for suppression of mast cell degranulation.

---

**Fig. 2** Effect of tocotrienol (T3) on allergic dermatitis (AD) in NC/Nga mice. Scratching behavior (A), dorsal skin specimens (B), serum histamine levels (C), and serum IgE levels (D) in control mice and in mice with or without administration of tocotrienol followed by PiCl treatment. The scratching behavior of control, AD, and AD + T3 mice were videotaped for 20 minutes without any research staff in the observation room. Playback of the video was used for counting scratching events. The dorsal skin specimens were stained with hematoxylin and eosin and observed using a microscope. Serum was obtained from each mouse and the levels of histamine and IgE were determined by ELISA. Values are expressed as the mean ± SE (n=12). \( \text{a, b} \) Values with different superscripts are significantly different (\( p < 0.05 \)).
Anti-allergic effect of tocotrienols

3.4 Effect of T3 on histamine release from RBL-2H3 cells

Release of histamine causes the allergy symptoms that are decreased by T3. The effect of T3 on histamine release from mast cells was examined in RBL-2H3 cells. T3 significantly suppressed histamine release from these cells (Fig. 4), with δ-T3 having the strongest effect and α-T3 the weakest. The order of blocking of histamine release was δ-T3 > β-T3 > γ-T3 > α-T3. These results indicate that histamine release from RBL-2H3 cells is reduced by T3.

3.5 Effect of T3 on cell viability and PKC activity in RBL-2H3 cells

To investigate the mechanism of the antidegranulation effect of T3, the effects of T3 on cell viability and PKC activity were measured. T3 did not influence the viability of RBL-2H3 cells (Fig. 5A), indicating that cytotoxicity is not the basis of the blocking of degranulation. In contrast, T3 significantly suppressed the activity of PKC, which plays an important role in degranulation of mast cells (Fig. 5B). δ-T3 had the strongest effect and α-T3 had the weakest, with the order of blocking of the PKC activity being δ-T3 > β-T3 > γ-T3 > α-T3. These results show that blocking of mast cell degranulation by T3 is associated with suppression of PKC activity.

3.6 Concentration of T3 isoforms in RBL-2H3 cells

The strengths of the effects of physiologically active substances often depend on the intracellular concentration. To examine differences in the antidegranulation effects of T3 isoforms, the cellular uptake of each isoform was measured. The intracellular level of δ-T3 was especially high (Fig. 6) and the order of uptake was δ-T3 > β-T3 > γ-T3 > α-T3. This order is consistent with the strengths of the respective antidegranulation blocking effects and suggests that inhibition of mast cell degranulation by T3 is dependent on cellular uptake.

4 DISCUSSION

AD is a typical allergic disease that depends on an antigen-antibody interaction. In patients with AD, the Th1/Th2 balance of T helper (Th) cell is biased to Th2 and an excessive level of IgE is produced. This IgE causes degranulation of mast cells in skin and a resultant discharge of physiologically active substances such as histamine. Histamine causes itching and induces scratching behavior, which then weakens the barrier function of the skin and causes dermal inflammation. Therefore, suppression of mast cell degranulation can reduce allergy symptoms such as itching, scratching, and dermal inflammation.

In this study, the effect of T3 on allergy symptoms was examined in the atopic dermatitis model NC/Nga mouse, in which AD can be induced by PiCl. Induction of AD causes increases in scratching behavior, dermal thickening, and serum histamine and IgE levels, and these changes were mostly reduced in mice that also received T3. These results clearly show that T3 reduces allergy symptoms in vivo. Many agents that suppress allergy symptoms exert the effect by suppressing production of IgE. However, T3 did not influence the serum IgE level, but still suppressed the serum histamine level and scratching behavior, indicating an action of T3 downstream from production of IgE. This suggests that the anti-AD effects of T3 involve suppression of degranulation of mast cells.

The effect of T3 on mast cell degranulation was examined in rat basophilic leukemia (RBL-2H3) cells. T3 significantly suppressed degranulation in these cells in a dose-dependent manner and also significantly reduced histamine release. These effects are likely to be the basis of T3 suppression of allergy symptoms. Mast cells are activated by interaction of an antigen and IgE, which causes degranulation. A variety of phosphorylating enzymes such as PKC take part in this process and activation of PKC promotes mast cell degranulation. Therefore, suppression of the activation of PKC should suppress degranulation. It has previously been shown that T3 suppresses PKC activity in the context of an anti-cancer effect. This suggests that a similar effect on PKC activity may be part of the mechanism of the antidegranulation effect of T3. In this study, we found that T3 significantly suppressed PKC activity in RBL-2H3 cells, which supports the idea that blocking of degranulation in these cells by T3 is associated with reduced PKC activity.

α-T3 did not suppress PKC activity in RBL-2H3 cells, but suppressed both degranulation and histamine release in these cells (Fig. 5). This suggests that T3 suppresses degranulation of mast cells through reduction of PKC activity and via other mechanism. T3 partitions into the cell mem-

Table 1 Vitamin E concentrations in skin of NC/Nga mice fed tocotrienol.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AD</th>
<th>AD+T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-T3</td>
<td>0.3 ± 0.0a</td>
<td>0.4 ± 0.0a</td>
<td>3.2 ± 0.1b</td>
</tr>
<tr>
<td>β-T3</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>γ-T3</td>
<td>0.4 ± 0.1a</td>
<td>0.3 ± 0.0a</td>
<td>27.9 ± 1.4a</td>
</tr>
<tr>
<td>δ-T3</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>α-TOC</td>
<td>10.6 ± 0.5</td>
<td>13.2 ± 0.9</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
<td>β-TOC</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>γ-TOC</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>δ-TOC</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=12). n.d.; not detect.

Values with different superscripts are significantly different (p < 0.05).
Fig. 3  Effects of tocotrienol (T3) and tocopherol (TOC) on degranulation from RBL-2H3 cells. IgE-sensitized RBL-2H3 cells were treated with α-T3 (A), β-T3 (B), γ-T3 (C), δ-T3 (D), and TOC (E), and then stimulated with DNP-BSA. Degranulation from RBL-2H3 cells was measured using a β-hexosaminidase assay. Data are expressed as the percentage of the result at 0 μM. Values are expressed as the mean±SD (n=6). a,b,c,dValues with different superscripts are significantly different (p < 0.05).
brane and changes the stiffness of the membrane, which may influence degranulation. However, this hypothesis requires further examination.

There were large differences in the antidegranulation effects of $\alpha$-T3, $\beta$-T3, $\gamma$-T3, and $\delta$-T3, with the order of the effects being $\delta$-T3 $>$ $\beta$-T3 $>$ $\gamma$-T3 $>$ $\alpha$-T3. The strength of the effect may depend on the intracellular concentration and it has been reported that $\delta$-T3 is most easily taken into cells, among the T3 isoforms. Consistent with this finding, examination of the cellular uptake of each T3 isoform in the current study showed that the intracellular level of $\delta$-T3 was especially high (Fig. 6). The order of uptake was $\delta$-T3 $>$ $\beta$-T3 $>$ $\gamma$-T3 $>$ $\alpha$-T3, consistent with the strengths of the blocking effect on degranulation. Therefore, these results suggest that the antidegranulation effect of T3 is dependent on cellular uptake. The differences in intracellular levels of the T3 isoforms may depend on structural differences among the isoforms, based on the different positions and numbers of methyl groups. $\delta$-T3 has the fewest methyl groups and this might permit easier cellular uptake.

In this study, an anti-allergic effect was shown only for T3, and not for TOC. T3 has several specific physiological functions and more easily enters the cell membrane and cytoplasm, compared to TOC. Thus, the physiological functions of T3 may occur because of the relatively high intracellular and cell membrane T3 concentrations. Further work is needed to examine this hypothesis.

T3 concentrations in the skin significantly increased after T3 administration, which suggests that T3 reached the skin of mice and acted on mast cells in the skin. The dose of T3 used in the study was 1 mg/day/mouse, which corresponds to 1.5-2.0 g/day in humans. Previous studies...
T3 and can be included in food products. However, a component in food products. Therefore, we suggest that T3 can be used as an antiallergic agent.

5 CONCLUSION
The results of this study provide the first evidence that T3 reduces AD by suppressing degranulation of mast cells. Topical application to the skin has been shown to reduce dermal inflammation caused by UV-B and similar application of T3 might also reduce AD. In contrast, TOC did not have this effect. Thus, the antidegranulation effect of T3 might be a feature of its physiologic activity, as well as antiangiogenic and hypocholesterolemic activities. These findings suggest the potential of T3 as an antiallergic agent.

ACKNOWLEDGMENT
This study was supported by The Uehara Memorial Foundation and The Iijima Memorial Foundation For The Promotion Of Food Science And Technology, Japan. The authors declare that there are no conflicts of interest.

References


26) Tsuzuki, T.; Kawakami, Y. Tumor angiogenesis suppression by alpha-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via peroxisome proliferator-activated receptor gamma. Carcinogenesis 29, 797-806 (2008).


