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Anti-allergic effect of strawberry extract

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ABSTRACT

We examined the anti-allergic effect of strawberry extract on human peripheral blood mononuclear cells (PBMCs) and atopic dermatitis model mice NC/NgaTndCrlj. The addition of strawberry extract suppressed total IgE production in the cedar pollen antigen Cry j 1-stimulated PBMCs. Flow cytometric analysis showed that strawberry extract decreased the rate of CD3⁺CD4⁺ helper T cells by 17.3% and increased the rate of CD3⁺CD8⁺ cytotoxic T cells by 19.7% in PBMCs. Moreover, the extract inhibited the expression level of GATA3 that is the master regulator of type 2 helper T cells (Th2) in human primary pan T cells isolated from PBMCs. Oral administration of strawberry extract lowered dermatitis scores and serum IgE levels in mice. In addition, it also decreased the GATA3 expression level in mouse blood cells. These results revealed that strawberry extract suppressed the severity of atopic dermatitis through the down-regulation of serum IgE by inhibition of Th2 differentiation.

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1. Introduction

In recent years, a number of functional foods that can improve health and reduce the risk of disease have been developed. Among them, anti-allergy foods have attracted much attention because allergy is a major modern health problem. Allergic diseases such as atopic dermatitis and asthma are hypersensitive immune responses, and they are known to be related to the balance between type 1 helper T (Th1) and type 2 helper T (Th2) cells (Abramovitis, 2005). Kan-

here et al. (2012) reported that Th1 and Th2 cells were differentiated from CD4⁺ T cells by expression of the master regulator, T-bet and GATA3, respectively. Th1 cells do not provide help for IgE synthesis, while Th2 cells induce IgE production on B cells (Romagnani, 1992). It is well known that IgE plays a critical role in acute hypersensitivity reactions. Cross-linking of high-affinity IgE receptor (FcεRI) with IgE and allergen induces the release of inflammatory mediators such as histamine, leukotrienes, cytokines and chemokines through degranulation (Corry & Kheradmand, 1999; Gould &

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Abbreviations: BSA, bovine serum albumin; CTL, cytotoxic T-lymphocyte; DEPC, diethyl pyrocarbonate; ELISA, enzyme-linked immunosorbent assay; FAD, flavin-adenine dinucleotide; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GATA3, GATA binding protein 3; HRP, horseradish peroxidase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IPSF, immunoglobulin production stimulating factor; IVI, in vitro immunization; MDP, muramyl dipeptide; NAD, nicotinamide adenine dinucleotide; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; SD, standard deviation; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SOCS3, suppressor of cytokine signaling 3; Th, helper T

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Sutton, 2008; Holgate, 1999). These mediators enhance allergic reaction including vasodilation, smooth muscle contraction, and mucosal edema. These findings strongly suggest that Th2 responses lead to allergic symptoms. Therefore, their suppression is key to the palliation of such symptoms.

There are a few reports on anti-allergic effects by food components. Stricinin can inhibit IgE production through the inhibition of IL-4 mediated signaling in B cells (Tachibana et al., 2001). Nagashio et al. (2013) showed that hesperidin inhibited skin lesions and IgE elevation. Epigallocatechin-3-O-(3'-methyl)-gallate can inhibit histamine release in vivo and in vitro (Maeda-Yamamoto, Ema, & Shibuichi, 2007). Ninomiya et al. (2010) reported that linocinnamarin and cinnamic acid in 'Nohime' strawberry inhibited antigen-stimulated degranulation through direct inhibition of spleen tyrosine kinase activation in vitro. We have recently reported that one of the IgE suppressors in strawberry is glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in vitro (Iwamoto et al., 2012). Furthermore, several studies have shown that strawberry has antioxidative activity, anti-tumour activity, anti-inflammatory activity and inhibitory effect on cognitive decline (Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Devore, Kang, Brereler, & Grodstein, 2012; Zhang, Seeram, Lee, Feng, & Herber, 2008), but its anti-allergic effect has not been fully understood as of yet. In this study, we examined the anti-allergic effect of strawberry extracts on human peripheral blood mononuclear cells (PBMCs) and atopic dermatitis model mice NC/NgaTndCrlj.

2. Materials and methods

2.1. Preparation of strawberry extract

Strawberries (*Fragaria × ananassa*) were purchased in Fukuoka prefecture, Japan in March 2011 and homogenized after the addition of equal weight of phosphate buffered saline (PBS) to extract components. The homogenate was centrifuged at 9400g at 4 °C for 60 min, the supernatant was filtrated through a 0.22 µm membrane (Millipore, Billerica, CA, USA) and used as the strawberry extract. Strawberry extract was sterilized by filtration for in vitro experiments, and concentrated up to fourfold by Amicon Ultra-15 10 K device (Millipore, Billerica, CA, USA) lyophilized for in vivo experiments.

2.2. Determination of protein and GAPDH levels

The protein concentration in strawberry extracts was determined using BIO-RAD protein assay (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (BSA) as a standard. The protein in the strawberry extract was added to IVI at 40 µg/ml and model mouse at 110 µg/mouse/day, respectively. The extracts were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and staining with Coomassie dye, and the band density of 39 kDa that equal to GAPDH molecular weight was quantified using NIH-Image system.

2.3. Cells and cell culture

Human PBMCs were separated from heparinized human blood of healthy donors by using Ficoll-Paque Plus (GE Healthcare,

Uppsala, Sweden), and human peripheral T cells were isolated from human PBMCs by using Easy sep Negative Selection Human T cell Enrichment Kit (Stem Cell Technologies, Vancouver, BC, Canada). The separated cells were stored at –85 °C in deep freezer.

To induce IgE production of PBMCs, in vitro immunization (IVI) was performed as described by Kawahara, Maeda-Yamamoto, and Hakamata (2000). Briefly, human PBMCs and separated T cells were cultured at a final density of 2.0×10^6 cells/ml in the 5% FBS and 10% human plasma containing ERDF medium (Kyokuto Pharmaceuticals, Tokyo, Japan) supplemented with 10 ng/ml of recombinant human IL-2, IL-4, and IL-6 (R&D systems, Minneapolis, MN, USA), 10 µg/ml of muramyl dipeptide (MDP; SIGMA, St. Louis, MO, USA) and 100 ng/ml of the cedar pollen antigen Cry j 1 (HAYASHIBARA, Okayama, Japan) with or without 5% (v/v) of strawberry extract. After 5 days, the GATA3 expression of T cells was examined by real-time PCR and after 10 days, total IgE concentration in the PBMCs supernatant was measured by an enzyme-linked immunosorbent assay (ELISA). Noteworthy, initial IgE production level was different between donors. All cells were cultured under humidified 5% CO₂/95% air atmosphere at 37 °C.

2.4. Measurement of IgE antibodies

Total IgE concentration in culture medium was measured by sandwich ELISA. Ninety-six well microplates were coated with anti-human IgE (Biosource, Camarillo, CA, USA) diluted with PBS. The antibody-coated wells were blocked with 1.0% BSA/PBS, and then, each sample was added to them. After washing with 0.05% Tween 20 containing PBS (TPBS) three times, biotin-conjugated anti-human IgE antibody (Biosource, Camarillo, CA, USA) and horseradish peroxidase (HRP)-conjugated streptavidin (Vector laboratories, Burlingame, CA, USA) were added to them. After washing with TPBS three times, a substrate solution (0.1 M citrate buffer (pH 4.0) containing 0.003% H₂O₂ and 0.3 mg/ml p-2, 2'-azino-di(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was added to them. After 15 min, the absorbance at 415 was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

2.5. Flow cytometric analysis

The PBMCs cultured under the IVI condition were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-human CD3 antibody as T cell marker and phycoerythrin (PE)-conjugated anti-human CD4 antibody as helper (Th) marker, anti-human CD8 antibody as cytotoxic T-lymphocyte (CTL) marker (BD Pharmingen, Franklin Lakes, NJ, USA) on ice in the dark for 30 min. After washing in 0.1% BSA/PBS three times, cells were resuspended in 0.1% BSA/PBS. The analysis of fluorescence-labeled cells was performed with a BD FACS-Vantage SE flow cytometer system (BD, Franklin Lakes, NJ, USA).

2.6. Real-time PCR

Total RNA was extracted from cultured cells using ISOGEN (Nippongene, Tokyo, Japan). The RNA extracted was

reverse-transcribed to cDNA using Thermoscript RT-PCR system (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed in 25 μ l reaction volume using 12.5 μ l of SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 9.5 μ l of diethyl pyrocarbonate (DEPC)-treated water, 1 μ l of cDNA synthesized and 10 μ M of the following appropriate primers:

human β -actin sense: 5'-AACTGGAACGGTGAAGGTGAC-3'
 antisense: 5'-ATGGCAAGGGACTTCCTGTAAC-3'
 human GAPDH sense: 5'-CTTCGCTCTCTGCTCCTCCTG-3'
 antisense: 5'-CGCCCAATACGACCAAATCGC-3'
 human GATA3 sense: 5'-TGTCTTCCCCTTCTTCTCTT TGC-3'
 antisense: 5ATGGCAAGGGACTTCCTGTAAC-3'
 mouse β -actin sense: 5'-TGGAATCCTGTGGCATCCATGAA AC-3'
 antisense: 5'-TAAAACGCAGCTCAGTAACAGTCCG-3'
 mouse GATA3 sense: 5'-TTATCAAGCCCAAGCGAAG-3'
 antisense: 5'-AGACCGGGTCCCCATTAG-3'.

For amplification, samples were heated 95 °C for 5 min and cycled 40 times at 95 °C for 30 s, 62.5 °C for 30 s and 72 °C for 30 s, and fluorescence intensity of amplification product was detected using MyiQ™ single color Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The size of amplified PCR products was confirmed by agarose gel electrophoresis. GATA3 expression was shown as the fold difference normalized to GAPDH or β -actin expression.

2.7. Continuous feeding of strawberry extract in NC/NgaTndCrJ mice and evaluation of severity in skin lesions

Male 7-week-old NC/NgaTndCrJ mice were purchased from Charles River Japan (Yokohama, Japan). These mice were housed under conditions of controlled temperature

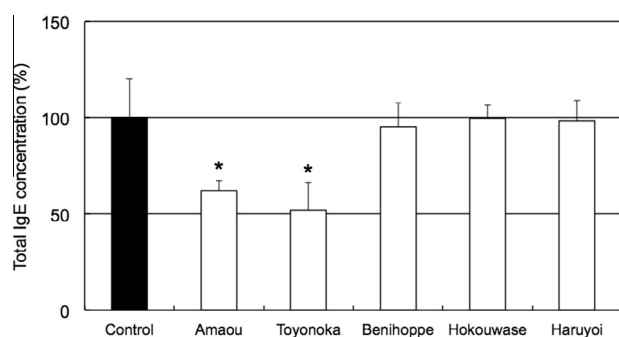


Fig. 1 – Suppressive effect of strawberry extracts on IgE production in IVI. Human PBMCs were cultured in 10% human plasma and 5% FBS containing ERDF medium supplemented with IL-2, -4, -6, MDP and Cry j 1 for 10 days. The amount of IgE produced in PBMCs was measured by ELISA. As a control, PBS only was added to the medium. Strawberry extracts were added to the medium at the final concentration of 5% (v/v). The result showed the average value of five independent measurements. Data are means $\pm$ SD ($n = 5$). Statistically significant differences from control were represented as * $p < 0.01$.

(25 $\pm$ 2 °C) and humidity (50 $\pm$ 13%) with 12 h/12 h of light/dark cycle. Food and tap water were provided *ad libitum*. They were divided into three groups (six mice in each group) and atopic dermatitis was induced according to pathogenic protocol of Charles River Japan.

All groups were topically applied with 5% picryl chloride (2,4,6-trinitrochlorobenzen) dissolved in ethanol/acetone (3:1, v/v) to the hair-removed chest and abdomen for the first day and with 1% picryl chloride dissolved in olive oil to the hair-removed back and ear once a week from the 4 day. Each group was administrated orally by a feeding tube with the following samples: control (4 mg of pectin/mouse/day that is equivalent amount of sugar in strawberry extract), 'Amaou' and 'Toyonoka' strawberry extract (17 mg/mouse/day until 10 weeks and 32 mg/mouse/day from the 11 week).

The severity of dermatitis was evaluated once a week. The development of (1) erythema/hemorrhage, (2) scarring/

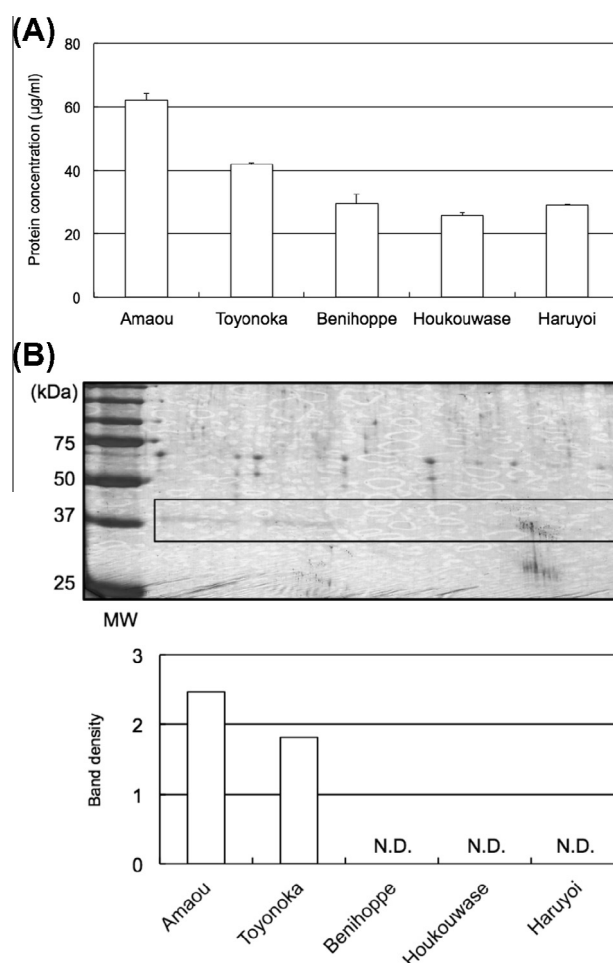


Fig. 2 – Protein and putative GAPDH levels in strawberry extracts. (A) The protein concentration in strawberry extracts was determined by the Bio-Rad protein assay with BSA as a standard. The result showed the average value of three independent measurements. Data are means $\pm$ SD ($n = 3$). (B) The extracts subjected to SDS-PAGE and staining with Coomassie dye, and the band density of 39 kDa quantified using NIH-Image system. N.D., Not detected.

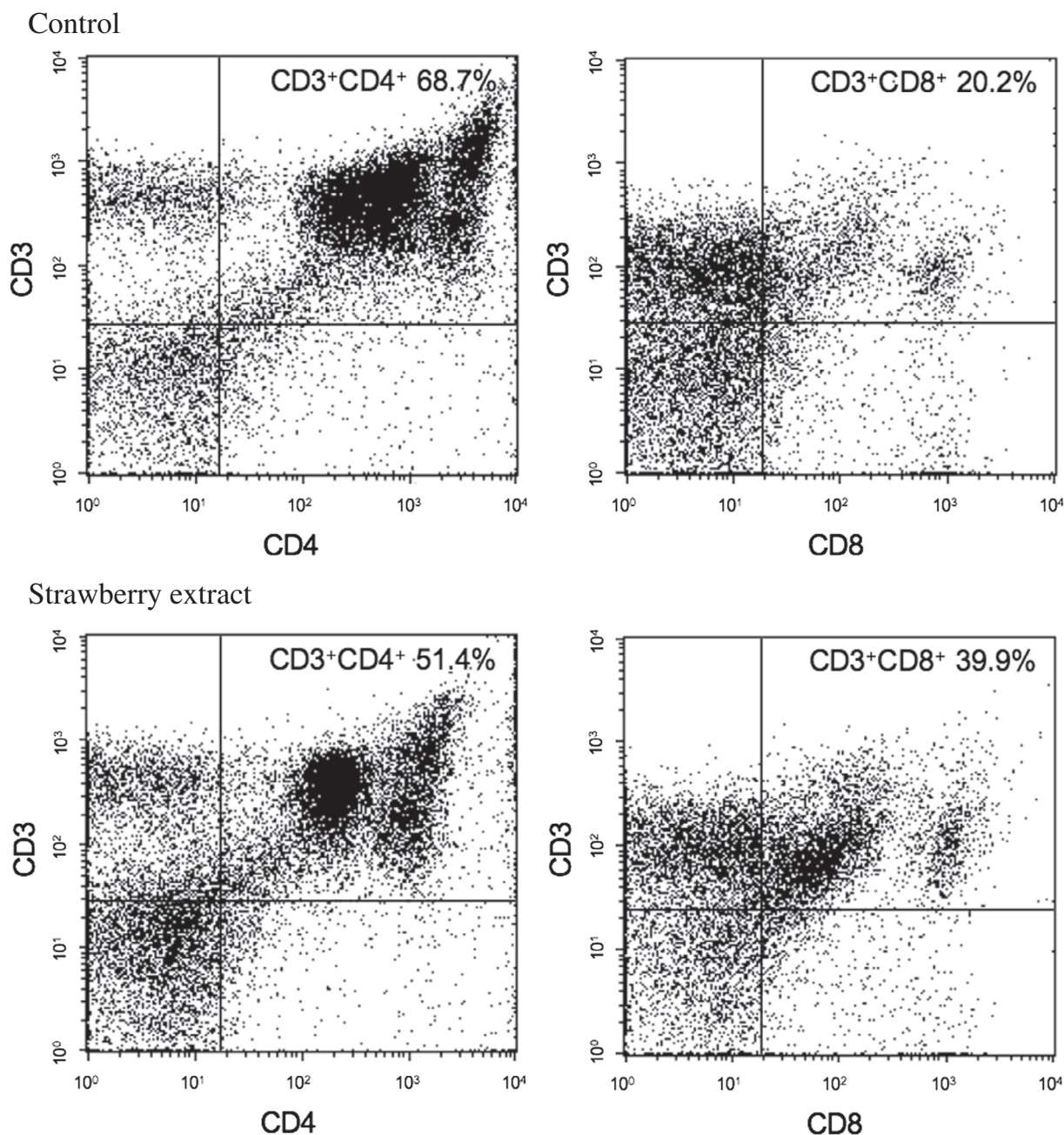


Fig. 3 – Effect of strawberry extract on cell population of PBMCs in IVI. Human PBMCs were cultured in 10% human plasma and 5% FBS containing ERDF medium supplemented with IL-2, -4, -6, MDP and Cry j 1 for 10 days. As a control culture, PBS only was added to the medium. ‘Toyonoka’ strawberry was added instead of PBS at the final concentration of 5% (v/v). The analysis of fluorescence-labeled cells was performed with a BD FACS Vantage SE flow cytometer system.

dryness, (3) edema, (4) excoriation/erosion was scored as 0 (none), 1 (mild, 0.25 cm²), 2 (moderate, 1 cm²), 3 (severe, 4 cm²). The sum of individual scores was taken as the dermatitis score. At 14 weeks after immunization, the level of IgE was measured in sera by ELISA. The mouse IgE antibody was measured using anti-mouse IgE (Biosource, Camarillo, CA, USA) and anti-mouse IgE HRP conjugated (Biosource, Camarillo, CA, USA). This experiment was undertaken in strict accordance with appropriate guideline for handling laboratory animals and the Law (No. 105) and Notification

(No. 6) of Japanese government for welfare of experimental animals.

2.8. Statistics

Data obtained are expressed as the means $\pm$ standard deviations (SD). The student's *t* test was used to assess the statistical significance of the difference against the control. Each value of **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 was considered to be statistically significant.

3. Results and discussion

3.1. Suppressive effect of strawberry extract on IgE production

The suppressive effects of strawberry extracts on the IgE production of PBMCs were examined. 'Amaou' and 'Toyonoka' significantly suppressed total IgE production by 38% and 48%, respectively (Fig. 1). But, strawberries such as 'Benihoppe' and 'Houkouwase', 'Haruyoi' did not suppress IgE production. Therefore, we focused on 'Amaou' and 'Toyonoka' strawberries in this study.

The protein and GAPDH levels in these extracts were examined. Because we previously demonstrated that a 39 kDa protein, that occupies mostly strawberry extract, is a novel IgE suppressor in strawberry (Iwamoto et al., 2012). As shown in Fig. 2(A), the protein concentration in the extracts with IgE suppressive activity was relatively higher than others. Fig. 2(B) shows the result of SDS-PAGE. There were no bands around a 39 kDa that represents GAPDH. The 39 kDa band in those extracts was also higher than others. Unfortunately, however, we cannot determine correctly the strawberry GAPDH level because there are no antibodies with specificity or cross-reactivity against it. From these results, it was suggested that specific strawberry species may have the IgE suppressive effect.

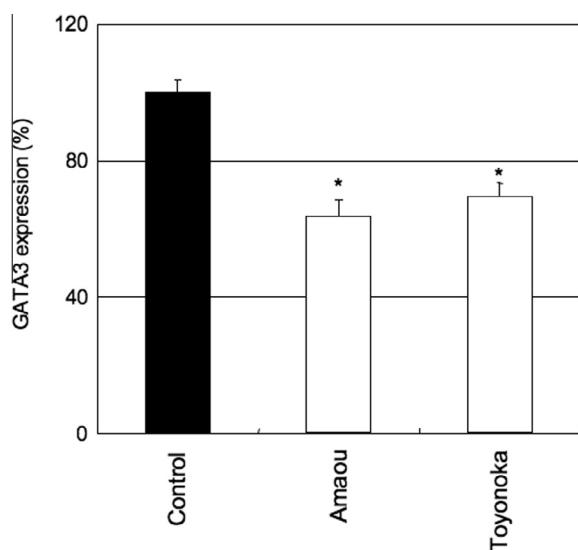


Fig. 4 – Inhibition of GATA3 expression by strawberry extract in human T cells. Human primary T cells were cultured in 10% human plasma and 5% FBS containing ERDF medium supplemented with IL-2, -4, -6, MDP and Cry j 1. After 5 days cultivation, GATA3 expression level of T cells was measured by real-time PCR using MyiQ5™ Real-Time PCR Analysis System. 'Amaou' and 'Toyonoka' strawberries were added to be 5% (v/v) to a control (PBS). The expression level of the GATA3 in control culture was estimated as 100%. The results showed the average value of three independent measurements. Data are means $\pm$ SD ($n = 3$). Statistically significant differences from control were represented as * $p < 0.01$.

3.2. Effect of strawberry extract on cell population

To investigate the effect of 'Toyonoka' strawberry extract on cell population of Th cells and CTLs, PBMCs cultured under the IVI condition were analyzed. Flow cytometric analysis showed that the rate of Th cells ($CD3^+CD4^+$) decreased by 17.3%, and CTLs ($CD3^+CD8^+$) increased by 19.7% in the strawberry extract containing culture (Fig. 3). Th2 of Th cells produces cytokines associated with IgE production such as IL-4, -5, -6, -10 and -13 (Skapenko et al., 2004). CTLs are involved in cell-mediated immunity and produce $IFN\gamma$ that can independently regulate Th1 development (Anderson, Schrama, Straten, & Becker, 2006; Bradley, Dalton, & Croft, 1996; Romagnani, 1997; Staeger, Gisch, & Kunz-Reske, 2003). These results suggest that strawberry extract suppressed IgE production by a decrease of Th cells and an increase of CTLs.

3.3. Inhibitory effect of strawberry extract on GATA3 expression

The expressions of master regulator of Th2, GATA3 were examined in human peripheral blood T cells cultured with or without strawberry extract. Th2 cells were known to induce class switching to IgE in B cells by producing cytokine such as IL-4, -5 and -13, and they are involved in humoral immunity against extracellular pathogens (Zhu, Yamane, Cote-Sierra, Guo, & Paul, 2006). As the result of real-time PCR, GATA3 expression in the cells cultured with strawberry was decreased compared with that in the cells cultured with PBS (Fig. 4). This may show the correlation between decreased GATA3 expression and IgE suppression. In the previous study, we also confirmed that the IgE suppression by strawberry extract was accompanied with a decreased expression of the suppressor of cytokines signaling-3 (SOCS3) that regulate the onset and maintenance of Th2 in PBMCs cultured under IVI condition (Mitsuda, Inoue, Nishino, Inoue, & Kawahara, 2010). These results suggest that the IgE suppressive effect of strawberry may be caused by inhibition of Th2 cell differentiation or proliferation.

3.4. Oral administration of strawberry extract suppresses skin manifestation of NC/NgaTndCrlj mice

The effect of strawberry extract on severity of atopic dermatitis and IgE concentration *in vivo* was examined. The NC/NgaTndCrlj mouse develops skin inflammation-like atopic dermatitis by immunizing with picryl chloride. Six mice were given orally strawberry extract or pectin as control until 14 weeks. There was no significant difference in body weight between three groups during the experimental period (data not shown). The severity of dermatitis was evaluated weekly. As a result, the difference of dermatitis score between control and strawberry extract (17 mg/mouse/day) was a few until 10 weeks (data not shown). However, the intake of strawberry extract (32 mg/mouse/day) remarkably inhibited the appearance of skin symptoms. The representative clinical feature of NC/NgaTndCrlj mice skin at 4 weeks after the start of strawberry extract administration is shown in Fig. 5(A). In addition, at 3 weeks after the start of strawberry extract

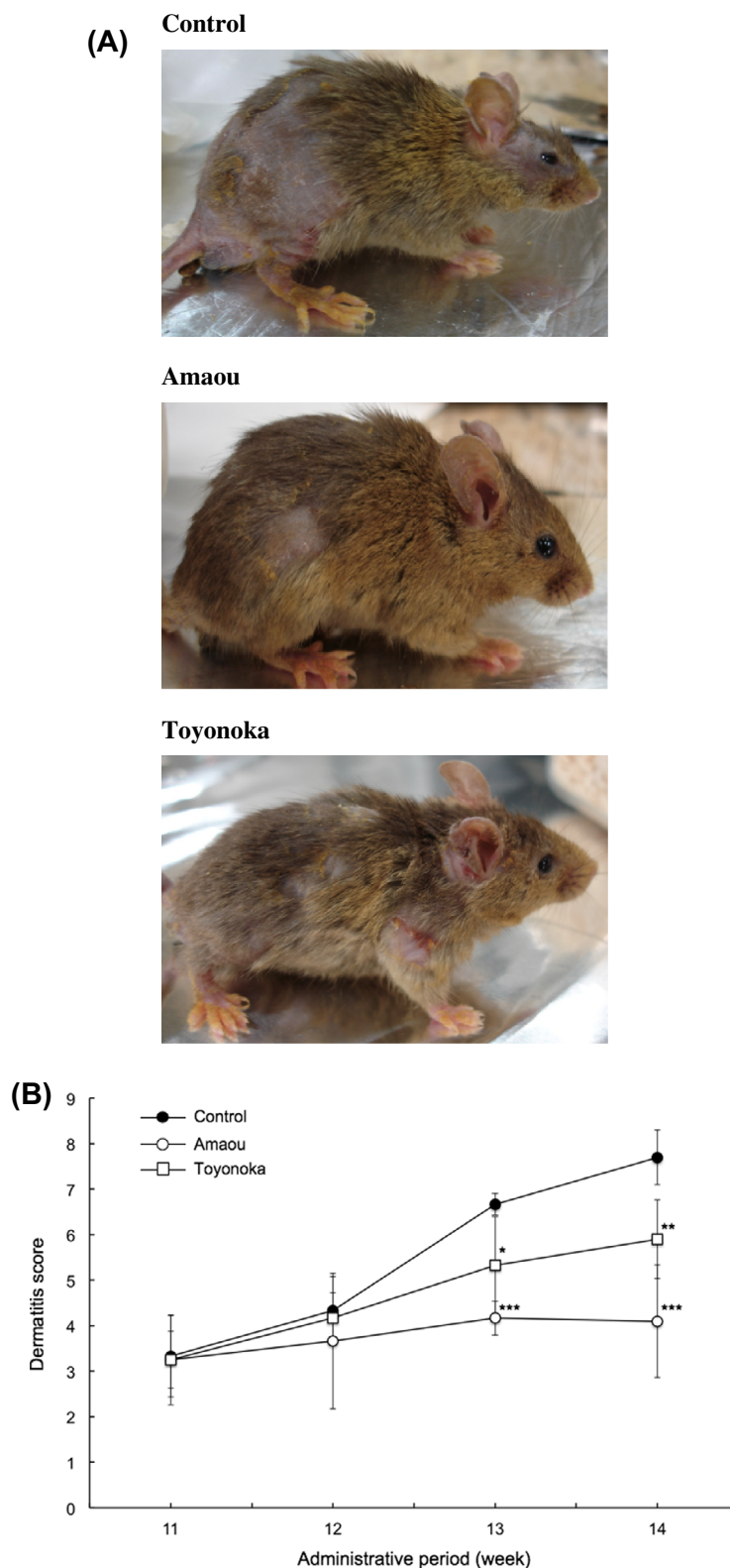


Fig. 5 – Effect of strawberry extract on clinical scores of skin symptoms. Strawberry extract was orally administrated to seven-week-old NC/NgaTndCrJ mice at 17 mg/mouse/day until 10 weeks. These mice were provided with strawberry extract at 32 mg/mouse/day from the 11 week. (A) Representative clinical feature of NC/NgaTndCrJ mouse. The photographs show mice at 14 weeks from the start of experiment. (B) Weekly dermatitis scores were measured weekly. The results showed the average value from the six independent mouse. Data are means $\pm$ SD ($n = 6$). Statistically significant differences from control were represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

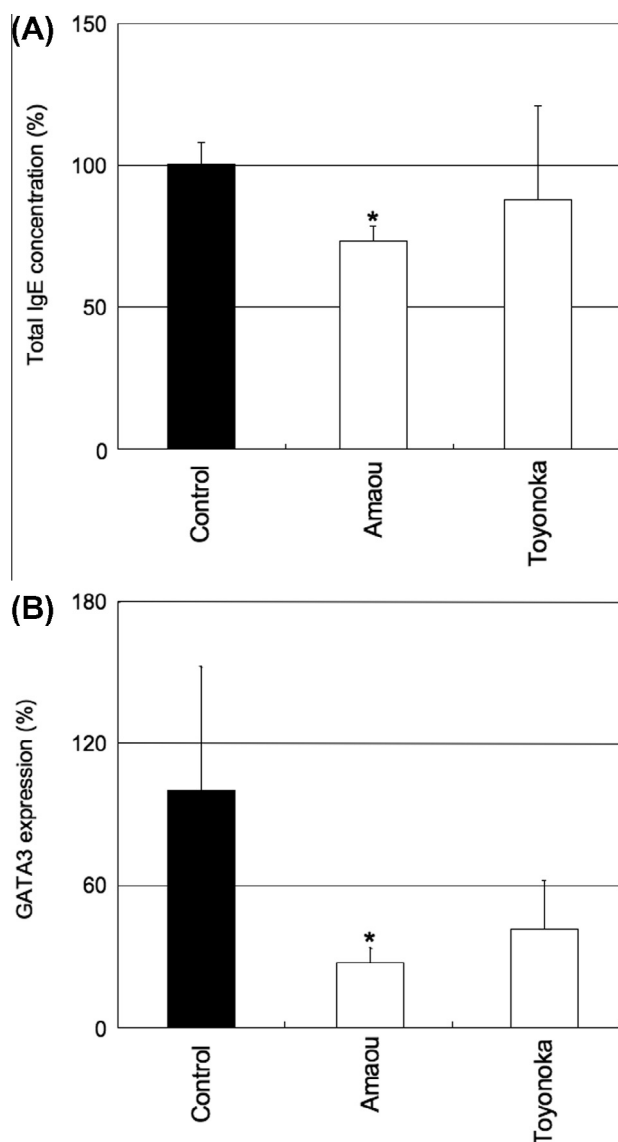


Fig. 6 – Effect of strawberry extract on serum IgE levels and mRNA expression of GATA3 in NC/NgaTndCrlj mice. 4 weeks after oral administration of strawberry extract at 32 mg/mouse/day, the effect of strawberry extract were examined. (A) The concentration of IgE in mice serum was measured by ELISA. The amount of the serum IgE in control mouse was estimated as 100%. (B) GATA3 expression was measured by real-time PCR using MyiQ5™ Real-Time PCR Analysis System. The expression level of the GATA3 from peripheral blood cells in control mouse was estimated as 100%. The results showed the average value from the three independent mouse of representative dermatitis score. Data are means $\pm$ SD ($n = 3$). Statistically significant differences from control were represented as * $p < 0.05$.

administration, the ameliorative effects were significant (Fig. 5(B)). The mean score of skin severity from the extract of ‘Amaou’ and ‘Toyonoka’ groups gradually decreased and reached 4.1 ± 1.2 and 5.9 ± 0.9 , respectively, at four weeks compared with the control group which showed a gradual

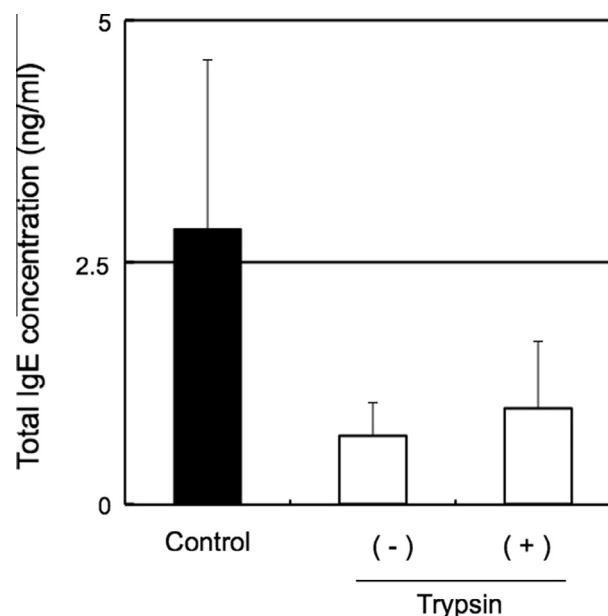


Fig. 7 – Effect of GAPDH digested by trypsin on IgE production in IVI. One $\mu\text{g/ml}$ of GAPDH ultrafiltered with a 10 kDa cut membrane was trypsinized by 10 $\mu\text{g/ml}$ of trypsin for 1 h. After trypsin treatment, the effect of digestion on IgE production in IVI was evaluated. Black bar represents solvent only as a control. The result showed the average value of three independent measurements. Data are means $\pm$ SD ($n = 3$).

increase and remained high score (7.7 ± 0.6). Furthermore, IgE levels of mouse serum were also decreased by ‘Amaou’ and ‘Toyonoka’ extracts (Fig. 6(A)). In particular, ‘Amaou’ extract significantly suppressed elevation of IgE level. It was reported that the skin dermatitis score of NC/NgaTnd and NC/Nga mouse correlated with plasma total IgE level (Matsumoto, Itakura, Tanaka, Fujisawa, & Matsuda, 2001; Matsumoto et al., 2002; Tanaka, Muto, Jung, Itai, & Matsuda, 2007). These findings support our data that the dermatitis score of ‘Amaou’ was more suppressed than ‘Toyonoka’ because ‘Amaou’ remarkably decreased elevation of IgE levels. Moreover, oral administration of strawberry inhibited the expression levels of the Th2 master regulator, GATA3 in NC/NgaTndCrlj mice blood cells (Fig. 6(B)). Kim, Cheong, Park, and Lee (2011) showed that skin manifestation and serum IgE level in NC/Nga mouse were improved by inhibition of Th2 development. These findings suggest that strawberry extract may suppress dermatitis score by inhibition of the IgE levels and Th2 differentiation, and it may have anti-allergic activity in vivo as well as in vitro.

In a previous study, we demonstrated that GAPDH is at least one IgE suppressor in strawberry (Iwamoto et al., 2012), but it is difficult to purify it in large amounts. Therefore, we cannot determine the IgE suppressive effect the purified GAPDH on NC/NgaTndCrlj mice. Thus, the effect of trypsin-digested GAPDH on IgE production was examined in IVI (Fig. 7). This result suggests that IgE suppressive activity of GAPDH was caused by its peptides. Sugahara and Sasaki (1998) reported that NAD⁺-binding region of GAPDH may

involve in the increase of IgM production. IgM are known to be class-switched to IgE by IL-4 stimulation in activated B lymphocyte. In our case, *in vitro* results correspond to those *in vitro*. Therefore, GAPDH peptides, in particular, the NAD⁺-binding region may influence the process of IgE class-switching. Interestingly, Kleiger and Eisenberg (2002) demonstrated that the V/IXGX₁₋₂GXXGXXXG/A motif is best for predicting whether a protein sequence adopts the Rossmann fold and bindings to FAD or NAD(P). The NAD⁺-binding region of strawberry GAPDH includes this motif and GXXXA sequence that stabilize it was not digested by trypsin including a basic amino acid (data not shown). Thus, strawberry GAPDH may be more suitable for IgE suppression than others. Moreover, Madureira et al. (2007) showed that microbial GAPDH induced an increase of immunoglobulin such as IgM secreting cells and sera levels of IL-10 in C57BL/6 mice. IL-10 is known to be a major down-regulator of inflammatory responses (Moore, de Waal Malefyt, Coffman, & O'Garra, 2001). Jeannin, Lecoanet, Delneste, Gauchat, and Bonnefoy (1998) reported that IL-10 suppressed IgE production and enhanced IgG4 production by inhibition of IgE class-switching. These studies indicate that GAPDH may suppress IgE production through an inhibition of IgE class-switching. These also supported our results that the specific species of strawberries that included GAPDH at the higher level than other suppressed IgE production by inhibition of Th2 differentiation.

In addition, Yano et al. (2007) reported that dietary flavones suppressed the up-regulation of serum IgE through the suppression of Th2-type immune response in BALB/c mice, and Park, Shin, Seo, and Kim (2007) showed that anthocyanin attenuated the development of asthma by down-regulating Th2 cytokines in a murine asthma model. Flavonoid and anthocyanin are abundantly included in strawberry. The combination of GAPDH with them may be highly effective in improving allergic symptoms such as atopic dermatitis.

Strawberry is popular all over the world. However, it has also the allergen Fra a 1. Therefore, certain individuals must be careful and alert around the allergy. Our results provide a possibility that continuous ingestion of strawberry may be able to palliate allergic symptoms.

4. Conclusions

Strawberry extract suppressed IgE production and it may be caused by inhibition of Th2 differentiation *in vitro*. The IgE suppressive effect by strawberry extract was also confirmed in sera from atopic dermatitis model mice, and oral administration of strawberry extract inhibited skin manifestation. Moreover, it inhibited GATA3 expression in mouse peripheral blood cells. However, further studies are needed to understand the suppressive mechanism of strawberry components. In therapy of allergy, anti-allergic drugs are usually used to improve atopic manifestation. They inhibit the release and activity of chemical mediators from mast cells, but produce occasionally harmful side effects. Our study may lead to the development of functional foods to relieve allergic conditions using strawberry.

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