We examined the effect of a crude hot-water extract (HW) of quince (Cydonia oblonga Miller) fruit on type I allergy in vivo and in vitro. The oral administration of the quince HW-added diet to NC/Nga mice for 63 d showed a significant decrease in the development of atopic dermatitis-like skin lesions under conventional conditions. The concentration of IgE in the serum collected from mice fed with quince HW was also lowered in a dose-dependent manner. Moreover, we found that quince HW inhibited the release of β-hexosaminidase from rat basophilic leukemia cell line RBL-2H3 after a 24-h treatment. The quince HW fraction of less than 3 kDa reduced the mRNA expression of the high-affinity IgE receptor (FcεRI) γ subunit. These results suggest that quince HW had an inhibitory effect on type I allergy by suppressing IgE production and IgE-mediated degranulation.

Key words: quince; NC/Nga mouse; degranulation; IgE; high affinity IgE receptor (FcεRI)

Quince (Cydonia oblonga Miller) is a deciduous shrub originating in Central Asia. The fruit features a rich yellow and waxy surface. Although they are too hard to eat raw and have a strongly acidic and astringent taste, they exhibit an extremely pleasant aroma. Therefore, extracts of quince fruits have long been used for honeydew and liquors. With regard to the function of the quince fruit, a methanol extract and an aqueous acetone extract have been reported to exhibit antioxidantive, antiviral, antilucreative, and antimicrobial activities. In addition, some products made from the hot-water extract (HW) of quince fruits are empirically known to be effective for treating sore throats and to relieve allergic symptoms. Among them, there has recently been an increasing incidence of type I allergic disorders from food- and air-borne allergens worldwide. This type of allergy is recognized as an IgE-mediated immediate hypersensitive reaction against environmental allergens and is believed to be associated with an elevated level of IgE antibodies produced against the allergens. Cross-linking of the high-affinity IgE receptor (FcεRI) on the surface of mast cells or basophils by IgE and a multivalent antigen triggers the exocytotic release of many chemical mediators including histamine. These mediators cause an immediate allergic reaction.

Since these allergic reactions are caused by a series of events, the inhibition of many steps is considered to attenuate the allergic symptoms. In fact, many researchers have reported the distinct mechanism-based anti-allergic effect of fruits. Given the effectiveness of quince HW-supplemented products against sore throats and coughs, quince HW can also be expected to have some sort of inhibitory effect against the type I allergic process.

We examined in the present study the anti-allergic effects of quince HW in vivo and in vitro. To evaluate the effect in vivo, we investigated an oral administration of HW to atopic model mice. Furthermore, we evaluated the anti-degranulation effect of HW by using an in vitro culture of RBL–2H3 cells, frequently used as a model of mast cells and basophils for their availability in IgE-mediated degranulation.

Materials and Methods

Preparation of the quince sample. Quince fruits were purchased from a fruit and vegetable market in Minowa-machi. They were cut into approximately 10 × 10 mm-size pieces including the peel, pulp and seeds, and then boiled in hot water for 1 h. After filtering the extract through a layer of cloth, the filtrate was centrifuged at 5,000 × g for 30 min. The resulting supernatant was concentrated to 450 ml in an RE 400A-W rotary evaporator (Yamato, Tokyo, Japan) at 40 °C, and freeze-dried to obtain quince HW.

Fractionated quince HW was prepared by passing the HW solution through Vivaspin 500 apparatus (MW cut off of 3,000; Vivascience, Hannover, Germany). After centrifugation at 5,000 × g, the lower (>3,000) fraction was collected. The unpassed contents were obtained as >3,000 fraction by re-dissolving the same volume of DW.

Mice. Three-week-old male NC/Nga mice were purchased from Japan SLC (Shizuoka, Japan) and housed at 23 ± 3 °C under a 12-h light/dark cycle. All animal protocols used in this study were approved by the Committee for Animal Experiments of Shinshu University.

Feeding and sampling. The mice were acclimatized with free access to an AIN-93M standard rodent diet (Clea Japan, Tokyo, Japan) and water for 7 d under conventional conditions. They were then assigned three groups consisting of five mice each on the basis of their body weights and fed on the following diets ad libitum for 8 weeks: control diet (AIN-93M), 2.5% quince HW-supplemented diet, and 5.0% quince HW-supplemented diet (Table 1). Throughout the experimental
Table 1. Composition of the Test Diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet (%)</th>
<th>2.5% quince HW-added diet (%)</th>
<th>5.0% quince HW-added diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>46.57</td>
<td>46.57</td>
<td>46.57</td>
</tr>
<tr>
<td>Milk casein</td>
<td>14.00</td>
<td>14.00</td>
<td>14.00</td>
</tr>
<tr>
<td>a-corn starch</td>
<td>15.50</td>
<td>15.50</td>
<td>15.50</td>
</tr>
<tr>
<td>Granulated sugar</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Purified soybean oil</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Avicel (cellulose powder)</td>
<td>5.00</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Quince HW extract</td>
<td>—</td>
<td>2.50</td>
<td>5.00</td>
</tr>
<tr>
<td>Mineral mix (AIN-93-MX)</td>
<td>35.00</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93VX)**</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>t-Cystine</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Coline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Tertiary butyl hydroquinone</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*Mineral mix: calcium carbonate anhydrous, 35.7%; potassium phosphate, monobasic, 25.0%; sodium chloride, 7.4%; potassium sulfate, 4.66%; potassium citrate, tri-potassium monohydrate, 2.8%; magnesium oxide, 2.4%; ferric citrate, 0.60%; zinc carbonate, 0.165%; manganese carbonate, 0.063%; cupric carbonate, 0.01% potassium iodate, 0.001%; sodium selenite anhydrous, 0.001025%; ammonium paramolybdate, 4 hydrate, 0.00795%; sodium meta-silicate, 9 hydrate, 0.145%; Chromium potassium sulfate, 12 hydrate, 0.0275%; boric acid, 0.00815%; sodium fluoride, 0.00635%; nickel carbonate, 0.00318%; lithium carbonate, 0.00174%; ammonium vanadate, 0.000866%; powdered sucrose, 20.908%.

**Vitamin mix: nicotinic acid, 0.30%; Ca pantothenate, 0.32%; pyridoxine-HCl, 0.07%; thiamin-HCl, 0.06%; riboflavin, 0.06%; folic acid, 0.02%; d-biotin, 0.10%; vitamin B12, 0.25%; vitamin E, 1.50%; vitamin A, 0.04%; vitamin D3, 0.02%; vitamin K1, 0.0075%; powdered sucrose, 97.252%.

period, the body weights of the mice were recorded weekly. Blood samples were collected from the abdominal aorta of the mice under ether anesthesia on d 63 to measure the serum IgE concentration. The serum total IgE level was measured by an enzyme-linked immunosorbent assay (ELISA). The serum total IgE level was measured by an enzyme-linked immunosorbent assay (ELISA). The serum total IgE level was measured by an enzyme-linked immunosorbent assay (ELISA).

Assessment of the clinical skin severity. The clinical severity of atopic dermatitis (AD)-like skin lesions of the five signs and symptoms (erythema/hemorrhage, edema, hair loss, dryness, and scaling) of the mice was assessed every week. The total clinical score for the severity is defined as the sum of individual scores graded as 0 (subclinical), 1 (mild), 2 (moderate), 3 (severe), and 4 (very severe) as described previously. Additionally, photographs of representative mice exhibiting average clinical symptoms in each group were taken after the majority of mice had shown AD-like skin lesions.

Measurement of the IgE level. The serum total IgE level was measured by an enzyme-linked immunosorbent assay (ELISA). Briefly, blood samples collected in assist tubes were stored overnight at 4 °C for blood clot formation. After clotting, the serum was separated by centrifugation at 300 × g for 30 min and stored at −80 °C until needed. The IgE concentration of the serially diluted sera was measured with a mouse IgE ELISA kit (Bethyl Laboratories, Montgomery, TX) according to the manufacturer’s protocol.

Cells. The rat basophilic leukemia cell line, RBL-2H3, was purchased from the Health Science Research Resources Bank (Osaka, Japan). The cells were maintained in an RPMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, TX), 2 mM L-glutamine (Invitrogen, Carlsbad, CA), 100 IU/ml of penicillin (MP Biomedicals, Irvine, CA), and 100 μg/ml of streptomycin (Wako, Osaka, Japan) in a humidified atmosphere of 5% CO₂/95% air at 37 °C.

Beta-hexosaminidase release assay. To evaluate IgE-mediated degranulation, a β-hexosaminidase release assay was employed as described previously. RBL-2H3 cells (5 × 10⁵ cells/ml) were treated with quince HW at 0, 50, 100, and 200 μg/ml in a 35-mm dish (Nunc, Roskilde, Denmark) for 24 h. After the treatment, the cells were harvested and washed twice with a HEPEs-Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 2.7 mM KCl, 0.5 mM NaH₂PO₄, 1.0 mM CaCl₂, and 10 mM HEPEs at pH 7.3) containing 0.1% bovine serum albumin. The washed cells were suspended in the HEPEs-Tyrode buffer and inoculated into a 96-well tissue culture plate (Becton Dickinson, NJ) at a concentration of 1 × 10⁶ cells/ml. The cells were sensitized or not with 1 μg/ml of the mouse monoclonal anti-dinitrophenyl (DNP) IgE antibody (clone SPE-7; Sigma, St. Louis, MO) for 2 h and exposed to 10 ng/ml of DNP-labeled human serum albumin (Sigma) at 37 °C for 30 min. The supernatants of the culture (50 μl) was transferred into a 96-well microtiter plate (Becton Dickinson) and mixed with 50 μl of 1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide (Sigma) in a 0.1 mM citrate buffer (pH 4.5) at 37 °C for 1 h. After the incubation, the reaction was terminated by adding 200 μl of a 0.1 M sodium carbonate buffer (pH 10.0). The absorbance of the mixture at 405 nm was measured with a microplate reader (model 550; Bio-Rad, Hercules, CA). The relative value of the β-hexosaminidase activity was calculated by the following formula: inhibition (%) = [(1 – (T – N)/(P – N))] × 100, where T, P, and N respectively represent the absorbance of equal portions of the supernatant from quince HW-treated cells upon degranulation, control (untreated) cells upon degranulation, and control cells without the degranulation treatment with antigen alone. In parallel with this assay, the growth and viability of the cells in the presence of absence of quince HW for 120 h was evaluated by counting the cells with a hematocytometer after staining with trypan blue.

Reverse transcription polymerase chain reaction (RT-PCR). RBL-2H3 cells were cultured at 1 × 10⁶ cells/ml in an RPMI 1640 medium containing 10% FBS with or without 50, 100, or 200 μg/ml of quince HW for 24 h. After this cultivation, total RNA was extracted from the cells by using the TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The RNA (1 μg) was reverse-transcribed into cDNA with 1 μg of random (DNP) primers, and 20 μg/ml of M-MLV reverse transcriptase (Invitrogen) at 42 °C for 50 min, using a PTC-200 DNA Engine (MJ Research, Waltham, MA). All PCRs were performed with a Taq PCR Core kit (Quagen, Chatsworth, CA) and 10 μg/ml of primers by using PTC-200. The primer sequences used in this study were as follows: FcɛRI α (sense), 5′-GGGACATCCCTCTCAAAATG-3′; FcɛRI α (antisense), 5′-GGGTACAGACATCTTCTG-3′ (product size 729, accession number M17153); FcɛRI β (sense), 5′-GGGACACATCTT- GTTCTCC-3′; and FcɛRI β (antisense), 5′-GACATCTCCTACACT- GAATG-3′ (product size 564, accession number M29223); FcɛRI γ (sense), 5′-GGGAGAGAAATGGCTAGT-3′; FcɛRI γ (antisense), 5′-GCTCTGCTATATCCTGGATG-3′ (product size 381, accession number BC160864); and β-actin (sense), 5′-TGATG- CTCCTGCTGTCACAC-3′; and β-actin (antisense), 5′-ACAGAGATGCTCCGGCTCAGG-3′ (product size 592, accession number BC031664). The following program was used: 94 °C for 1 min, 64 °C for 1 min, and 72 °C for 2 min, 30 cycles for FcɛRI α; 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, 28 cycles for FcɛRI β; 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, 30 cycles for FcɛRI γ; 94 °C for 30 s, 64 °C for 1 min, and 72 °C for 1 min, 18 cycles for β-actin. After the amplification, 10 μl of the reaction mixture was electrophoresed on 2% agarose gel and the PCR products were visualized as bands by ethidium bromide staining for 20 min. Gel images were captured with a Printraph AE-6911FX device (Atto, Tokyo, Japan).

Statistical analysis. Statistical analysis of the data employed a one-tailed and two-tailed Student’s t-test for in vivo and in vitro studies, respectively. A p-value of less than 0.05 was accepted as being statistically significant.

Results

Skin severity
Hot-water extraction yielded 173 g of quince HW from approximately 3,200 g of quince fruits. The effect of the oral administration of the quince HW-added diet...
The anti-allergic effect of quince fruit has not been reported despite its useful application against sore throats and coughs. In this study, we revealed the novel inhibitory effects of quince HW on AD-like symptoms in NC/Nga mice. As shown in Fig. 1, the development of skin lesions in NC/Nga mice is shown in Fig. 1. Throughout the experimental period, no significant difference in the change of average body weight was apparent among the groups (Fig. 1A). Five AD-like signs and symptoms appeared clinically on the face, ear, nose, neck, and dorsal skin of mice fed with the control diet at around d 21 or later of the experiment and developed gradually with aging. The skin severity scores of the 2.5% and 5.0% quince HW-fed group tended to be lower than the control group. The 5.0% quince HW-fed group showed no apparent skin lesions until d 42 and significantly (p < 0.01) alleviated grades of dermatitis on d 42 and d 49 compared to the control diet group (Fig. 1B). The alleviated clinical features of the symptoms were observed, particularly on the face and ears of the mice in both quince HW-added diet groups compared with the control group (Fig. 1C). The scratching behavior of the 5.0% quince HW diet group tended to be less than that of the control group (data not shown).

Serum IgE level

Serum was separately collected from control diet group, 2.5% quince HW-added diet group, and 5.0% quince HW-added diet group on d 63. The levels of IgE in the serum were as follows: control group, 1635 ± 289; 2.5% quince group, 1300 ± 226; and 5.0% quince group, 994 ± 205 ng/ml (Fig. 2). Quince HW reduced the IgE level in a dose-dependent manner. In particular, the 5.0% quince HW-added diet group showed a highly significant value (p < 0.01).

Expression of FceRI subunits

The expression of FceRI subunits in RBL-2H3 is shown in Fig. 4. Treatment with quince HW showed no effects on the expression of the FceRI α and β subunits. In contrast, the expression of the γ subunit was notably reduced (Fig. 4A). The fraction of quince HW weighing less than 3 kDa showed reduced expression of the FceRI γ subunit, while that of more than 3 kDa had a negligible effect (Fig. 4B).

Discussion

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of skin severity was significantly reduced in the mice fed with the 5.0% quince HW-added diet. The serum IgE level of the quince HW-added diet groups was lowered in a dose-dependent manner (Fig. 2). NC/Nga mice are known to exhibit spontaneous development of dermatitis accompanied by IgE elevation with age under conventional circumstances. Many reports have demonstrated that an elevated level of IgE antibodies played an important role in the development of AD-like skin lesions in NC/Nga mice. Furthermore, elevation of the serum IgE level has been reported to be correlated with the degranulation of mast cells and eosinophils. Our results suggest that the oral administration of quince HW inhibited AD-like skin lesions by an unreported IgE-suppressing mechanism. However, it has also been reported that the development of some chronic AD-like
skin lesions in NC/Nga mice was independent of the plasma IgE level. These facts led us to further investigate the distinct inhibitory effect of quince HW on the development of allergic symptoms.

Figure 3 shows that the \( \beta \)-hexosaminidase release from RBL-2H3 cells was significantly reduced by treating with quince HW without affecting the proliferation and viability of the cells. The value for released \( \beta \)-hexosaminidase has frequently been used as an indicator for evaluating the extent of degranulation by mast cells or basophils, because this exoglycosidase is stored in the secretory granules of these cells and released in parallel with chemical mediators by FcεRI-mediated activation. The decreased \( \beta \)-hexosaminidase activity suggests that quince HW inhibited the degranulation process itself.

We further found that a 24-h treatment with quince HW reduced the expression of the FcεRI \( \gamma \) subunit (Fig. 4A). FcεRI is expressed exclusively on mast cells and basophils for triggering the degranulation. FcεRI is expressed in mice as a tetrameric molecule consisting of one \( \alpha \) chain, one \( \beta \) chain, and two disulfide-linked \( \gamma \) chains. IgE-binding and signal-transduction of the FcεRI molecule are performed by distinct subunits. The FcεRI \( \alpha \) subunit mostly extends out to the extracellular region, binding to the constant region of IgE with high affinity to begin a cascade of cellular events. The FcεRI \( \beta \) subunit amplifies the signal strength. The FcεRI \( \gamma \) subunit enables the stably expression of the FcεRI \( \alpha \) subunit on the cell surface and transfers the signal to induce degranulation via immunoreceptor tyrosine activation motifs (ITAM). Therefore, it is possible that reduced expression of the FcεRI \( \gamma \) subunit was a factor in the inhibition of degranulation by quince HW.

We characterized the active substance in quince HW for suppressing the FcεRI \( \gamma \) subunit. FcεRI \( \gamma \) subunit expression was suppressed by the filtered (<3 kDa) fraction (Fig. 4B), suggesting that a low-molecular-weight substance was involved in down-regulation of the FcεRI \( \gamma \) subunit. To date, many low-molecular-weight polyphenols have been reported as anti-allergic substances that have anti-inflammatory effect, including the inhibition of histamine release from mast cells. These low-molecular-weight polyphenols are therefore suggested to have anti-inflammatory activity which inhibit FcεRI \( \gamma \) chain expression and IgE production have been found in limited sources other than quince fruits and have a specific structure distinct from the above-mentioned quince fruit-derived polyphenols. Other unknown polyphenolic or other substances may be involved in the suppressive effect of quince HW on type I allergic symptoms.

In conclusion, we found two anti-allergic effects of quince HW \textit{in vivo} and \textit{in vitro}. The inhibitory effects of quince fruit on both the IgE elevation in allergic mice and FcεRI \( \gamma \) chain expression in mast cells and basophils have not been reported in previous studies. Although further detailed studies are necessary to understand the anti-allergic mechanism of quince HW, our results suggest that quince HW would be applicable as a functional food for alleviating type I allergic symptoms.

\textbf{References}