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Kaempferol Isolated from *Nelumbo nucifera* Stamens Negatively Regulates FcεRI Expression in Human Basophilic KU812F Cells

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Mast cells and basophils perform important functions as pivotal effector cells in IgE-mediated allergic reactions. KU812F cells, a human basophilic cell line isolated originally from chronic myelocytic leukemia, express a high affinity receptor of IgE, FcεRI. Kaempferol was extracted and isolated from a methanolic extract of flavonoid-rich *Nelumbo nucifera* stamens. In the present study, the inhibitory effects of kaempferol on FcεRI expression in human basophilic KU812F cells was examined. Flow cytometric analysis revealed that FcεRI expression on the cell surface was suppressed in a concentration-dependent manner when the cells were cultured with kaempferol. Moreover, RT-PCR analysis showed that the mRNA levels for FcεRI α- and γ-chains were reduced as the result of kaempferol treatment in a concentration-dependent manner. Kaempferol showed its suppressive effects on intracellular Ca^{2+} concentration and histamine release from anti-FcεRI α-chain antibody-stimulated cells in a concentration-dependent manner. These observations indicate that kaempferol may exert antiallergic effect *via* downregulation of FcεRI expression and degranulation.

Keywords: Kaempferol, *Nelumbo nucifera* stamens, FcεRI, intracellular Ca^{2+}, histamine, KU812F

Mast cells and basophils express a high affinity IgE receptor, FcεRI, on their cell surfaces, and perform a pivotal function as effector cells in IgE-mediated allergic reactions [13, 14]. The aggregation of FcεRI by allergen-specific IgE antibody *via* the binding of multivalent allergens or anti-FcεRI antibodies triggers the release of a variety of chemical mediators, including histamine, prostaglandins, and leukotrienes from activated mast cells and basophils, resulting in allergic responses such as asthma, atopic dermatitis, and allergic rhinitis [4, 18]. FcεRI molecules on mast cells and basophils function as a tetrametic receptor containing one α-, one β-, and two disulfide-linked γ-chains. Among the three subunits of FcεRI, the α-chain is a specific component of FcεRI and mostly extends out into the extracellular region and binds directly to the Fc portion of the IgE antibody with high affinity [8]. Thus, the inhibition of FcεRI may result in an attenuation of the IgE-mediated allergic reaction.

Nelumbo nucifera Gaertn is a perennial aquatic plant of the Nymphaeceae family, and is distributed throughout Asia, the Middle East, and Egypt. All parts of *N. nucifera*, including leaves, flowers, stamens, embryos, and rhizomes, have been used as traditional medicines for the treatment of diarrhea, gastritis, insomnia, nervous prostration, and as a hemostatic [17, 31]. This plant has pharmacological properties including hepatoprotective, antimicrobial, antibacterial, anti-HIV, antioxidant, antihyperlipidemic, antiobesity, antidiabetic, and antihypertensive effects. In particular, *N. nucifera* stamens are flavonoid-rich, and have a variety of physiological properties, including antioxidative, antiinflammatory, and antidiabetic activities [3, 11, 12, 17]. Flavonoids are its important constituents that belong to a group of natural substances with variable phenolic structures found in fruits, vegetables, grains, flowers, tea, and wine [10, 19].

Recently, a host of studies on the antiallergic activities of food components have been conducted, and flavones chrysin and apigenin, as well as green tea catechin, (−)-epigallocatechin-3-O-gallate (EGCG), have been demonstrated to exert antiallergic effects *via* suppression of FcεRI expression [6, 7, 32]. However, the regulation of FcεRI expression by kaempferol has not yet been studied previously. In the current study, we determined whether kaempferol isolated from *N. nucifera* stamens exerted an inhibitory effect on FcεRI expression in human basophilic KU812F cells.

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Table 1. Primer sequences used in this study.

Name		Sequences
FcεRI α-chain	Sense	5'-CTTAGGATGTGGGTTCAGAAGT-3'
	Antisense	5'-ACAGTGGAGAATACAAATGTCA-3'
FcεRI β-chain	Sense	5'-TAATTCTTCATAAAGACGATCATCNGG-3'
	Antisense	5'-TATGCCTTTGTTTTGGAACAATGGTGTG-3'
FcεRI γ-chain	Sense	5'-TAGGGCCAGCTGGTGTTAATGGCA-3'
	Antisense	5'-GATGATTCCAGCAGTGGTCTTGCT-3'
G3PDH	Sense	5'-GCTCAGACACCATGGGGAAGGT-3'
	Antisense	5'-GTGGTGCAGGAGGCATTGCTGA-3'

Materials and Methods

Materials

Kaempferol was isolated from methanol extract of the stamens of *Nelumbo nucifera*. The anti-human FcεRI α-chain antibody, CRA-1, was purchased from Kyokuto (Tokyo, Japan). Mouse IgG antibody was purchased from Biosources (Burlingame, CA, U.S.A.). Anti-mouse IgG fluorescence isothiocyanate (FITC) antibody was purchased from Jackson ImmunoResearch Lab. (Baltimore, PO, U.S.A.). RPMI-1640 medium, fetal bovine serum (FBS), antibiotics, and antimycotics were purchased from Gibco BRL (Gaithersburg, MD, U.S.A.). Trizol reagent was purchased from Invitrogen (Carlsbad, CA, U.S.A.). Oligo $(dT)_{15}$ primer, MMLV reverse transcriptase, Go*Taq* DNA polymerase, and Celltiter 96 Aqueous One Solution Cell Proliferation Assay were obtained from Promega (Madison, WI, U.S.A.). All other reagents, including hydroxyethyl piperazinylethanesulfonic acid (HEPES), L-glutamine, histamine, and *o*-phthalaldehyde (OPA) were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.).

Cell Culture, Treatment, and Stimulation

KU812F cells were maintained in an RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 10 mM HEPES buffer, antibiotics, and antimycotics, and cultured at 37°C in a humidified atmosphere with 5% CO_2, and passaged every 3–4 days. The cells were incubated for 24 h in serum-free RPMI-1640 medium with different concentrations of kaempferol.

Cell Viability

Cell viability was assayed *via* Celltiter 96 Aqueous One Solution Cell Proliferation Assay in accordance with the manufacturer's instructions. KU812F cells were seeded on 96-well plates at a density of 2.5×10^4 cells/well and incubated with serum-free media in the presence of various concentrations of kaempferol. After 24 h of treatment, 100 μl of 10 times-diluted reagents was added and incubated for 4 h. The colored formazan products were then measured at 490 nm using a microplate reader (Molecular Devices, VersaMax, Sunnyvale, CA, U.S.A.). Relative cell viability was calculated and compared with the absorbance of untreated cells.

Determination of Cell Surface FcεRI Expression

FcεRI expression on the cell surface was measured *via* indirect immunofluorescence and flow cytometry. In brief, KU812F cells (1×10^6 cells) were treated with different concentrations of kaempferol, and incubated with 10 μg/ml of CRA-1 on ice for 60 min. The cells were then stained with 20 μg/ml of anti-mouse IgG FITC antibody on ice for 60 min, followed by washing with ice-cold PBS for flow cytometry (Beckman Coulter; EPICSXL, Fullerton, CA, U.S.A.). As a negative control, a mouse IgG antibody (10 μg/ml) was used instead of CRA-1. The percentage of FcεRI-positive cells was calculated with an arbitary cutoff position of 2%, as determined by the negative control.

Determination of FcεRI α, β, and γ Chains mRNAs

FcεRI subunit mRNA levels were determined *via* RT-PCR. Thus, total cellular RNA was extracted from the treated cells using Trizol reagent in accordance with the manufacturer's instructions. For cDNA synthesis, 1 μg of total RNA was reverse-transcribed using an oligo $(dT)_{15}$ primer and MMLV reverse transcriptase. The resultant cDNA samples were subjected to PCR amplification in the presence of specific sense and antisense primers. Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal control, and the primer sequences are provided in Table 1. The PCR was conducted as follows: 94°C, 30 sec denaturing; 55°C, 30 sec annealing; and 72°C, 1 min extension: and subjected to 18 and 23 cycles for FcεRI α- and β-, and γ-chain genes, respectively. The amplified PCR products were visualized *via* 1% agarose gel electrophoresis and ethidium bromide staining, and then analyzed using a Molecular Imager Gel Doc XR System (Bio-Rad, Hercules, CA, U.S.A.).

Measurement of Intracellular Ca^{2+}

Intracellular Ca^{2+} concentration was measured using the calcium reactive fluorescence probe Fura 2-AM. The KU812F cells were treated with different concentrations of kaempferol suspended in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 1.8 mM $CaCl_2$) and incubated with 2.0 μM Fura 2-AM at 37°C for 30 min. The cells were then washed three times, resuspended in fresh buffer, and stimulated with 10 μg/ml of CRA-1. The Fura 2 fluorescence was monitored with a microplate fluorescence reader (BioTek, FLx800; Winooskin, VT, U.S.A.) at an excitation wavelength of 360 nm and an emission wavelength of 528 nm.

Histamine Measurement

Histamine content was assessed *via* a spectrofluorometric assay [27]. The treated cells were stimulated with 10 μg/ml of CRA-1 for 30 min. After centrifugation, 1 N NaOH and 0.2% OPA were added to the supernatant and the mixtures were incubated on ice for 40 min. The reaction was terminated *via* the addition of 3 N HCl, and the fluorescence intensity was measured using a microplate

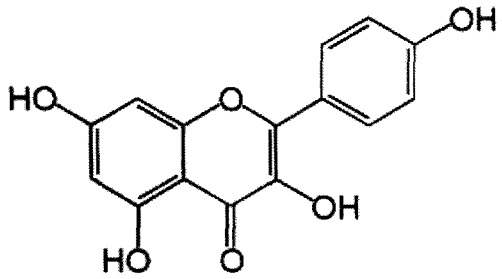

Fig. 1. Chemical structure of kaempferol.

fluorescence reader (BioTek, FLx800; Winooskin, VT, U.S.A.) at an excitation wavelength of 360 nm and an emission wavelength of 450 nm.

Statistical Analysis

The statistical differences between the control and kaempferol groups were determined *via* the Student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Cell Viability

In this study, human basophilic KU812F cells were treated with different concentrations of kaempferol (Fig. 1) isolated from *Nelumbo nucifera* stamens, in order to determine the range of nontoxic concentration. As shown in Fig. 2, kaempferol exerted no toxic effects at a concentration range of 0–35 μM.

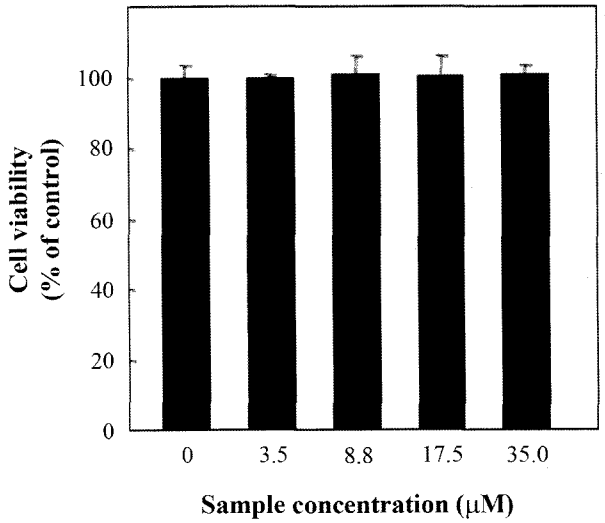

Fig. 2. Cytotoxic effect of kaempferol in KU812F cells.
KU812F cells were cultured for 24 h in the presence of different concentrations of kaempferol, and the cell viabilities were determined *via* MTS assay. Each determination was made in triplicate and data are expressed as means±SD.

Effects on Cell Surface FcεRI Expression

Mast cells and basophils express a high affinity IgE receptor, FcεRI, on their cell surface, and perform a crucial function in IgE-mediated allergic reactions [2]. KU812F cells are a human basophilic cell line originally isolated from chronic myelocytic leukemia, and express FcεRI [15]. In order to assess the inhibitory effect of kaempferol on the cell surface expression of FcεRI, KU812F cells were treated with different concentrations of kaempferol for 24 h under serum-free conditions, and the cell surface expression of FcεRI was assessed *via* indirect immunofluorescence and flow cytometry using CRA-1. FcεRI expression on the cell surface was reduced from 32.5% to 30.6%, 27.8%, 21.0%,

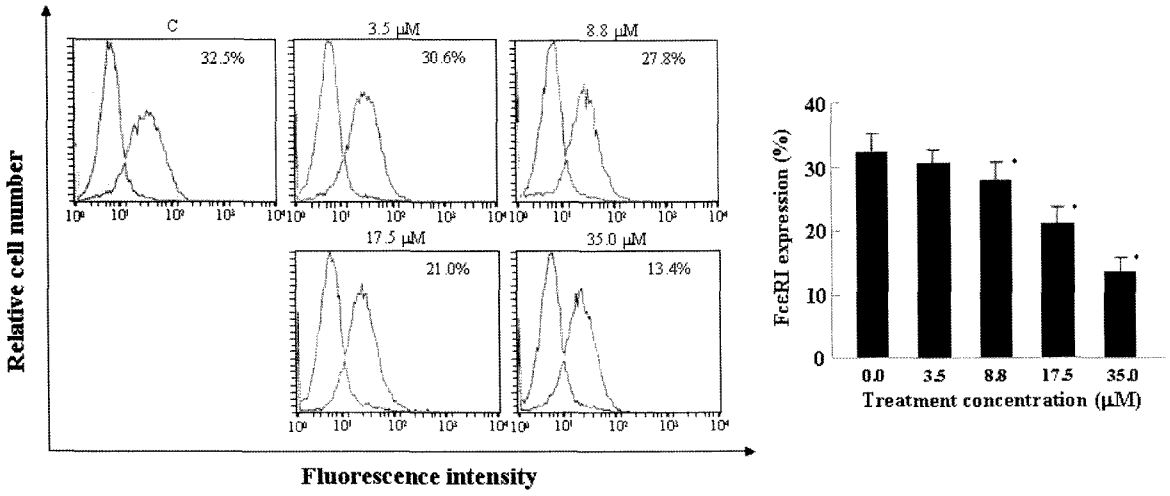

Fig. 3. Effects of kaempferol on cell surface FcεRI expression.
KU812F cells were cultured for 24 h in the presence of different concentrations of kaempferol. The cells were then incubated with CRA-1 followed by staining with anti-mouse IgG FITC antibody. The stained cells were then analyzed *via* flow cytometry. Each value represents the mean±SD of three different experiments. *Values are significantly different from the control ($*p<0.05$).

and 13.4% when treated with kaempferol at 0, 3.5, 8.8, 17.5, and 35.0 μM, respectively (Fig. 3).

Effects on FcεRI α-, β-, and γ-Chains mRNA Expression

In order to confirm the inhibitory effects of kaempferol on FcεRI gene expression, the mRNA levels of the FcεRI subunits were measured *via* RT-PCR using total cellular RNA prepared from KU812F cells treated with different concentrations of kaempferol. The mRNA for the FcεRI α- and γ-chains of non-treated cells was clearly detected; however, the corresponding mRNA of the kaempferol-treated cells was shown to be reduced (Fig. 4). On the other hand, no β-chain mRNA was detected under these conditions.

FcεRI is a tetrameric molecule composed of one α-, one β-, and two γ-chains. The α-chain is expressed in FcεRI-positive cells and is essential for functioning of the cell surface receptor for IgE [8, 22]. The gene expression of the FcεRI subunits was determined by the mRNA level via RT-PCR and was shown to be downregulated by kaempferol. These results indicate that the suppression of cell surface FcεRI expression in the presence of kaempferol was due to reduction of total cellular FcεRI subunit gene expression. Nishiyama *et al.* [21–24, 29] reported that the gene expression of the FcεRI α-chain is known to be regulated by at least two transcription factors; GATA and Elf-1 in rodents and mammals, including humans. Further studies on the regulation of the signal transcription of the gene encoding for the FcεRI α-chain are needed for better understanding of the mechanisms involved in the suppression of FcεRI expression by kaempferol.

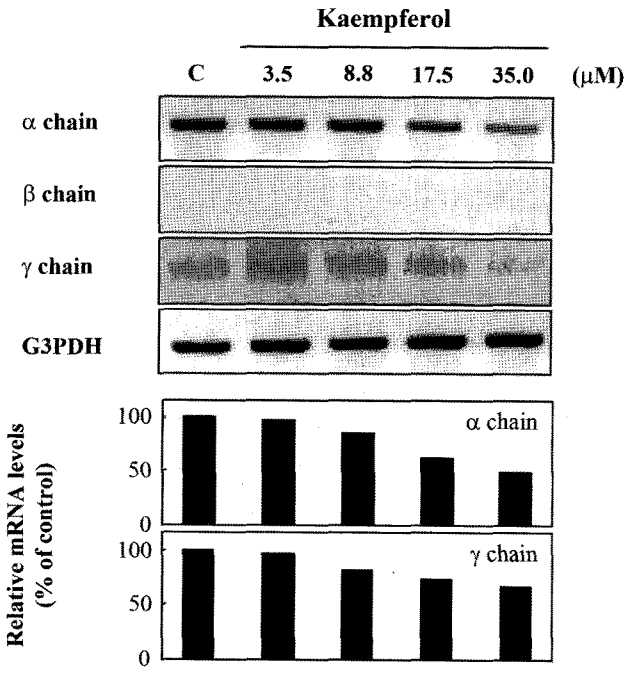

Fig. 4. Effects of kaempferol on FcεRI α-, β-, and γ-chains mRNA expression.
KU812F cells were treated with different concentrations of kaempferol, and FcεRI α, β, γ, and G3PDH mRNA levels were analyzed *via* RT-PCR.

Effects on FcεRI-Mediated Intracellular Ca^{2+} Concentration

Intracellular Ca^{2+} is important for the induction of mast cells and basophils degranulation occurring *via* FcεRI cross-linking. In order to determine the effects of kaempferol on intracellular calcium influx, KU812F cells were labeled with a calcium-specific fluorescence probe, Fura 2-AM. In the kaempferol-treated cells, the intracellular Ca^{2+} concentration in the CRA-1-stimulated cells was reduced in a concentration-dependent manner (Fig. 5). FcεRI cross-linking activates downstream signaling cascades, including intracellular Ca^{2+} influx, which is required for degranulation [16, 30]. We demonstrated that kaempferol treatment concentration-dependently inhibited FcεRI-mediated intracellular Ca^{2+}. Moreover, FcεRI cross-linking activates protein tyrosine kinases including Syk, Lyn, Fyn, and BTK, and the phosphorylation of numerous proteins in mast cells and basophils [1, 5, 9, 20, 26]. Therefore, in order to better understand the inhibitory mechanism of FcεRI expression *via* kaempferol, further studies regarding the regulation of the protein tyrosine kinases are needed.

Effects on FcεRI-Mediated Histamine Release

The aggregation of FcεRI by anti-FcεRI antibody or multivalent IgE-antigen complexes initiates a cascade of biochemical events that result in degranulation, induce the secretion of inflammatory mediators, and contribute to allergic responses [16, 25, 30]. Mast cells and basophils are granulated cells and are degranulated when the cells are

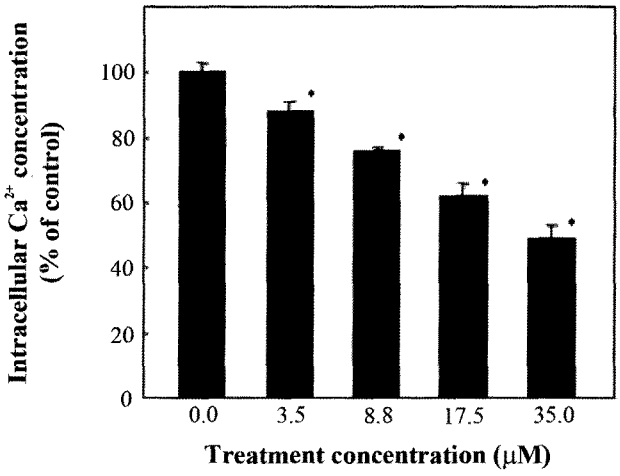

Fig. 5. Effects of kaempferol on intracellular Ca^{2+}.
The treated KU812F cells were incubated with Fura 2-AM and stimulated with CRA-1. Intracellular Ca^{2+} concentration was spectrofluorometrically determined. Each value represents the mean±SD of three different experiments. *Values are significantly different from the control ($*p<0.05$).

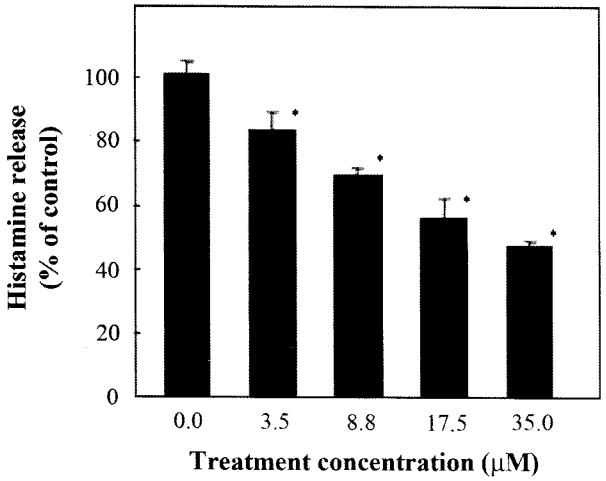

Fig. 6. Effects of kaempferol on FcεRI-mediated histamine release. The treated KU812F cells were stimulated with CRA-1, and histamine content was spectrofluorometrically determined. Each value represents the mean±SD of three different experiments. *Values are significantly different from the control (*$p<0.05$).

activated *via* the aggregation of FcεRI as the result of treatment with anti-FcεRI antibody or the IgE-antigen complexes [28]. In order to assess the inhibitory effects of kaempferol on degranulation, histamine release was measured from KU812F cells treated with different concentrations of kaempferol, and stimulated with CRA-1, and the histamine released from these cells was assessed. As shown in Fig. 6, FcεRI-mediated histamine release was reduced in a concentration-dependent manner.

In summary, the antiallergic effects of kaempferol isolated from the stamens of *Nelumbo nucifera* were analyzed, in an attempt to search for a biologically active antiallergic medicinal source. These results provide the first experimental evidence to suggest that kaempferol inhibits FcεRI expression in human basophilic KU812F cells, and that the inhibitory effects of kaempferol on cell surface FcεRI expression were associated with downregulation of the mRNA expressions of the α- and γ-chains of FcεRI and intracellular calcium influx, resulting in the release of a variety of inflammatory mediators. Our present results provide insight into the mechanism of FcεRI suppression by kaempferol, and these findings may contribute to the increasing body of knowledge regarding the search for potent antiallergic materials.

REFERENCES

1. Amoui, M., L. Drrova, P. Tolar, and P. Draber. 1997. Direct interaction of Syk and Lyn protein tyrosine kinases in rat basophilic leukemia cells activated *via* type Fc epsilon receptors. *Eur. J. Immunol.* **27:** 321–328.
2. Blank, U., C. Ra, L. Miller, K. White, H. Metzer, and J. P. Kinet. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature* **337:** 187–189.
3. Cho, E. J., T. Yokozawa, D. Y. Rhyu, S. C. Kim, N. Shibahara, and J. C. Park. 2003. Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical. *Phytomedicine* **14:** 544–551.
4. Drombrowicz, D., V. Flamand, K. K. Brigman, B. H. Koller, and J. P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor ε chain gene. *Cell* **75:** 969–976.
5. Eiseman, E. and J. B. Bolen. 1992. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. *Nature* **355:** 78–80.
6. Fujimura, Y., H. Tachibana, and K. Yamada. 2001. A tea catechin suppresses the expression of the high-affinity IgE receptor FcεRI in human basophilic KU812 cells. *J. Agric. Food Chem.* **49:** 2527–2531.
7. Fujimura, Y., H. Tachibana, M. Maeda-Yamamoto, M. T. Miyase, M. Sano, and K. Yamada. 2002. Antiallergic tea catechin, (−)-epigallocatechin-3-O-(3-O-methyl)-gallate, suppresses FcεRI expression in human basophilic KU812 cells. *J. Agric. Food Chem.* **50:** 5729–5734.
8. Hakimi, J. C., J. A. Seals, L. Kondas, W. Pettine, W. Danko, and J. Kochan. 1990. The α subunit of the human IgE receptor (FcεRI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* **265:** 22079–22081.
9. Hata, D., Y. Kawakami, N. Inagaki, C. S. Lantz, T. Kitamura, W. N. Khan, *et al.* 1998. Involement of Bruton's tyrosine kinase in FcεRI-dependent mast cell degranulation and cytokine production. *J. Exp. Med.* **187:** 1235–1247.
10. Huxley, R. R. and H. A. Neil. 2003. The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of propoective cohort diseases. *Eur. J. Clin. Nutr.* **57:** 904–908.
11. Jung, H. A., J. E. Kim, H. Y. Chung, and J. S. Choi. 2003. Antioxidant principles of *Nelumbo nucifera* stamens. *Arch. Pharm. Res.* **26:** 279–285.
12. Kim, H. K., H. R. Park, J. S. Lee, T. S. Chung, and H. Y. Chung. 2007. Down-regulation of iNOS and TNF-alpha expression by kaempferol *via* NF-kappaB inactivation in aged rat gingival tissues. *Biogerontology* **8:** 399–408.
13. Kinet, J. P. 1999. The high affinity IgE receptor (FcεRI): From physiology to pathology. *Annu. Rev. Immunol.* **17:** 931–972.
14. Kinet, J. P., U. Blank, A. Brini, M. H. Jouvin, H. Kuster, O. Mejan, and C. Ra. 1991. The high affinity receptor for immunoglobulin E: A target for therapy of allergic diseases. *Int. Arch. Allergy Appl. Immunol.* **94:** 51–55.
15. Kishi, K. 1985. A new leukemia cell line with Philadelphia chromosome characterized as basophile precursors. *Leuk. Res.* **9:** 381–390.
16. La Cour, B., P. Molgaard, and Z. Yi. 1995. Traditional Chinese medicine in treatment of hyperlipidaemia. *J. Ethnopharmacol.* **46:** 125–129.
17. Lim, S. S., Y. J. Jung, S. K. Hyun, Y. S. Lee, and J. S. Choi. 2006. Rat lens aldose reductase inhibitory constituents of *Nelumbo nucifera* stamens. *Phytother. Res.* **20:** 825–830.
18. Metzer, H. 1991. The high affinity receptor for IgE on mast cells. *Clin. Exp. Immunol.* **4:** 269–279.
19. Middleton, E. J. 1998. Effect of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.* **439:** 175–182.

20. Nadler, M. J., S. A. Matthews, H. Turner, and J. P. Kinet. 2000. Signal transduction by the high-affinity immunoglobulin E receptor FcεRI coupling form to function. *Adv. Immunol.* **76:** 325–355.
21. Nishiyama, C., M. Hasegawa, M. Nishiyama, K. Takahashi, Y. Akizawa, T. Yokota, K. Okumura, H. Ogawa, and C. Ra. 2002. Regulation of human FcεRI α-chain gene expression by multiple transcription factors. *J. Immunol.* **168:** 4546–4552.
22. Nishiyama, C. 2006. Molecular mechanism of allergy-related gene regulation and hematopoietic cell development by transcription factors. *Biosci. Biotechnol. Biochem.* **70:** 1–9.
23. Nishiyama, C., T. Ito, M. Nishiyama, S. Masaki, K. Maeda, N. Nakano, *et al.* 2005. GATA-1 is required for expression of Fc{epsilon} RI on mast cells: Analysis of mast cells derived from GATA-1 knockdown mouse bone marrow. *Int. Immunol.* **17:** 847–856.
24. Nishiyama, C., T. Yokota, K. Okumura, and C. Ra. 1999. The transcription factors Elf-1 and GATA-1 to cell-specific enhancer elements of human high-affinity IgE receptor α-chain gene. *J. Immunol.* **163:** 623–630.
25. Schwartz, L. B., R. A. Lewis, D. Selden, and K. F. Austen. 1981. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. *J. Immunol.* **126:** 1290–1294.
26. Siraganian, R. P., J. Zhang, K. Suzuki, and K. Sada. 2002. Protein tyrosine kinase Syk in mast cell signaling. *Mol. Immunol.* **38:** 1229–1233.
27. Shore, P. A., A. Burkhalter, V. H. Cohn, and H. C. Victor. 1959. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127:** 182–186.
28. Suzuki, Y., T. Yoshimura, T. Matsui, T. Inoue, O. Niide, S. Nunomura, and C. Ra. 2003. Fc(epsilon)RI signaling of mast cells activates intracellular production of hydrogen peroxide: Role in the regulation of calcium. *J. Immunol.* **171:** 6119–6127.
29. Takahashi, K., C. Nishiyama, and C. Ra. 1999. Transcriptional factors Elf-1 and GATA-1 bind to cell-specific enhancer elements of human-affinity IgE receptor alpha-chain gene. *J. Immunol.* **163:** 623–630.
30. Turner, H. and J. P. Kinet. 1999. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* **25:** 24–30.
31. Van Bergen, P. E., P. G. Hatcher, J. J. Boon, M. E. Collinson, and J. W. De Leeuw. 1997. Macromolecular composition of the propagule wall of *Nelumbo nucifera*. *Phytochemistry* **45:** 601–617.
32. Yono, S., H. Tachibana, and K. Yamada. 2005. Flavones suppress the expression of the high affinity IgE receptor FcεRI in human basophilic KU812F cells. *J. Agric. Food Chem.* **53:** 1812–1817.