

Down-Regulation of FcεRI Expression by *Houttuynia cordata* Thunb Extract in Human Basophilic KU812F Cells

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ABSTRACT Human basophilic KU812F cells express a high-affinity immunoglobulin (Ig) E receptor, FcεRI, which plays an important role in IgE-mediated allergic reactions. *Houttuynia cordata* Thunb (Family Saururaceae), which is rich in polyphenols, has been shown to have various physiological properties, including antiviral, antioxidative, anticancer, and anti-inflammatory activities. The effect of *H. cordata* extract on the expression of FcεRI in human KU812F cells was examined. Flow cytometric analysis showed that the FcεRI expression and the IgE binding activity were suppressed when the cells were cultured with *H. cordata* extract. Reverse transcription-polymerase chain reaction analysis showed that levels of the mRNAs for FcεRI α- and γ-chains were decreased by the treatment of *H. cordata* extract. Addition of *H. cordata* extract to culture medium was also observed to result in a reduction in the release of histamine from the cells. These results suggest that *H. cordata* extract may exert its anti-allergic activity through down-regulation of FcεRI expression and a subsequent decrease in histamine release.

KEY WORDS: • FcεRI • histamine • *Houttuynia cordata* Thunb • immunoglobulin E • KU812F cells

INTRODUCTION

HOUTTUYNIA CORDATA Thunb, also called Ur-Sung-Cho in Korea, is a perennial herb of the Saururaceae family. It has been used as a traditional medicine for treatment of cough, pneumonia, bronchitis, dysentery, dropsy, leukorrhea, uteritis, and eczema.¹ This plant contains essential oil components such as decanoyl acetaldehyde with antibacterial, antileukemic, antimutagenic, and antioxidative activities.^{2–5} Additional biological and pharmaceutical activities of *H. cordata* include inhibition of inflammation, passive cutaneous anaphylaxis, stem cell factor-induced HMC cell migration, and immunoglobulin (Ig) E production.^{6–10} However, the regulation of expression of FcεRI, a high-affinity IgE receptor, by *H. cordata* extract has not been studied.

FcεRI is found on the cell surface of mast cells and basophils and plays an important role in IgE-mediated allergic reactions.¹¹ A cross-linking of FcεRI molecules attached to allergen-specific IgE antibody by a binding of multivalent allergen triggers the release of a variety of chemical mediators such as histamine, prostaglandins, and leukotrienes

from activated mast cells and basophils, which results in allergic response including asthma, atopic dermatitis, and allergic rhinitis.^{12–15} The FcεRI molecule on mast cells and basophils is a tetrameric receptor composed of one α-, one β-, and two disulfide-linked γ-chains. Among the three subunits of FcεRI, the α-chain mostly extends out to the extracellular region, directly binds to the Fc portion of the IgE antibody with high affinity, and is a specific component of FcεRI.¹⁶ Thus, the down-regulation of FcεRI expression may lead to an attenuation of the IgE-mediated allergic reaction. Recently, many studies on anti-allergic activities of food components have been reported, and a green tea catechin, (–)-epigallocatechin-3-*O*-gallate, has been demonstrated to have anti-allergic activities through the suppression of FcεRI expression.^{17,18} In the present study, the suppressive effects of *H. cordata* extract on FcεRI expression in human basophilic KU812F cells were investigated.

MATERIALS AND METHODS

Reagents

Anti-human FcεRI antibody (CRA-1) was purchased from Kyokuto (Tokyo, Japan). Mouse IgG and fluorescein isothiocyanate (FITC)-conjugated anti-human IgE antibody were purchased from Biosources (Burlingame, CA). FITC-conjugated F(ab')₂ goat anti-mouse IgG was purchased from

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Jackson ImmunoResearch Laboratories (West Grove, PA). RPMI-1640 medium was purchased from Nissui Pharmaceutical Co. (Tokyo). Fetal bovine serum, antibiotics, and antimycotics were purchased from Gibco BRL (Gaithersburg, MD). TRIzol reagent, oligo(dT)₂₀ primer, and SuperScript II reverse transcriptase were purchased from Invitrogen (Carlsbad, CA). *Taq* DNA polymerase was purchased from Roche (Mannheim, Germany). All other reagents, such as HEPES, L-glutamine, histamine, and *o*-phthalaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of *H. cordata* extract

Fresh *H. cordata* that had been cultivated in Bosung, Republic of Korea, was rinsed with water, homogenized, and compressed to obtain juice. The juice was then naturally fermented with oligosaccharides and honey. The extract was lyophilized, stored at -20°C , and dissolved in phosphate-buffered saline before use.

Cell culture and determination of cytotoxicity

KU812F cells, a human basophilic cell line originally isolated from chronic myelocytic leukemia that expresses a high-affinity IgE receptor,¹⁹ were provided by Dr. Sanetaka Shirahata, Kyushu University, Fukuoka, Japan. The cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES buffer, and antibiotics and antimycotics, cultured at 37°C in a humidified atmosphere with 5% CO_2 , and passaged every 3–4 days. Cells were cultured in serum-free RPMI-1640 medium with various concentrations of *H. cordata* extract for 24 hours. Cell viability was evaluated spectrophotometrically at 490 nm using the Celltiter 96[®] Aqueous One Solution cell proliferation assay (Promega, Madison, WI).

Determination of cell surface Fc ϵ RI expression and binding activity using flow cytometric analysis

The expression of cell surface Fc ϵ RI and the binding activity of IgE were measured by indirect immunofluorescence and flow cytometry. In brief, KU812F cells (1×10^6 cells) were incubated with anti-human Fc ϵ RI α -chain antibody (10 $\mu\text{g}/\text{mL}$) for 60 minutes on ice. The cells were then stained with FITC-conjugated F(ab')₂ goat anti-mouse IgG (20 $\mu\text{g}/\text{mL}$) for 60 minutes on ice, followed by washing with ice-cold phosphate-buffered saline for flow cytometry. As a negative control, a mouse IgG antibody (10 $\mu\text{g}/\text{mL}$), replacing anti-human Fc ϵ RI α -chain antibody was used. The IgE and FITC-conjugated IgE were used to measure the binding activity of IgE and Fc ϵ RI.

Determination of Fc ϵ RI α , β , and γ mRNA levels using reverse transcription (RT)-polymerase chain reaction (PCR)

Total cellular RNA was isolated using TRIzol reagent according to the manufacturer's instruction. For cDNA syn-

thesis, 1 μg of total RNA was reverse-transcribed using an oligo(dT)₂₀ primer and SuperScript II 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 a control. Primer sequences used in this study were as follows: for the Fc ϵ RI α -chain, sense 5'-CTTAGGATGTGGGTTTCAGAAAGT-3' and antisense 5'-GACAGTGGAGAATACAAATGTCA-3'; for the Fc ϵ RI β -chain, sense 5'-TAATTCTTCATAAAGACGATCATC-NGG-3' and antisense 5'-ATATGCCTTTGTTTTGGAA-CAATGGTGTG-3'; for the Fc ϵ RI γ -chain, sense 5'-TAG-GGCCAGCTGGTGTAAATGGCA-3' and antisense 5'-GATGATTCCAGCAGTGGTCTTGCT-3'; and for G3PDH, sense 5'-GCTCAGACACCATGGGGAAGGT-3' and antisense 5'-GTGGTGCAGGAGGCATTGCTGA-3'. The PCR was performed as follows: 94°C , 30 seconds denaturing; 55°C , 30 seconds annealing; and 72°C , 1 minute extension, with 18 cycles for Fc ϵ RI α - and β -chain genes and 23 cycles for Fc ϵ RI γ -chain genes. The amplified PCR products were analyzed by agarose gel electrophoresis.

Histamine measurement

The histamine content was measured by a spectrofluorometric assay.²⁰ KU812F cells (1×10^6 cells/mL) were treated with different concentration of *H. cordata* extract for 24 hours. The treated cells were suspended in Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4 , 1 mM MgCl_2 , 12 mM NaHCO_3 , and 1.8 mM CaCl_2) and stimulated with 10 $\mu\text{g}/\text{mL}$ CRA-1 at 37°C for 30 minutes. After centrifugation, 1 N NaOH and 0.2% *o*-phthalaldehyde were added and incubated for 40 minutes on ice. The reac-

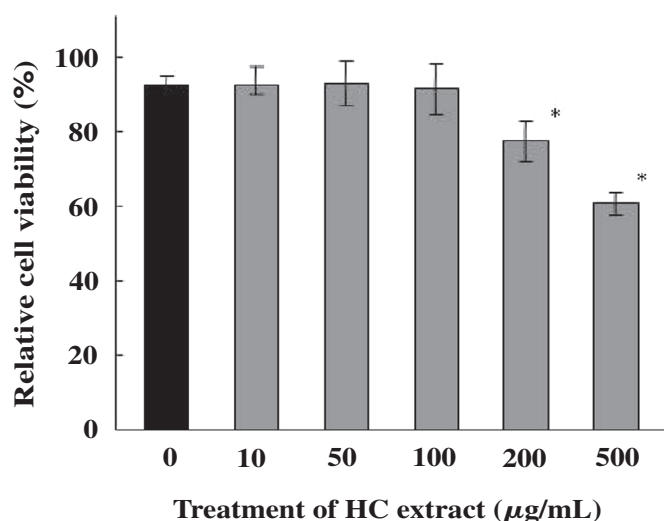


FIG. 1. Effects of *H. cordata* (HC) extract on cell viability. KU812F cells were cultured in the presence of different concentrations of HC extract for 24 hours, and the cell viabilities were determined by Celltiter 96 Aqueous One Solution cell proliferation assay. Each determination was made in triplicate, and data shown are mean $\pm$ SD values. *Values are significantly different from control ($P < .01$).

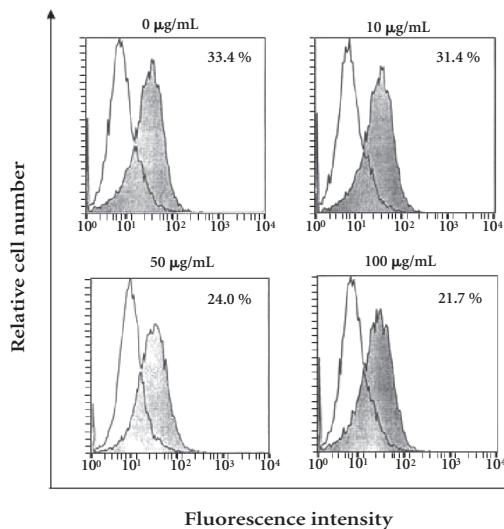


FIG. 2. Effects of *H. cordata* extract on cell surface expression of FcεRI. KU812F cells were cultured in the presence of different concentrations of *H. cordata* extract (0, 10, 50, or 100 μg/mL) for 24 hours. The cells were then incubated with anti-human FcεRI α-chain monoclonal antibody (CRA-1) followed by staining with FITC-conjugated F(ab')₂ goat anti-mouse IgG. Stained cells were then analyzed by flow cytometry. The percentage of FcεRI-positive cells was calculated with the arbitrary 2% cutoff position as determined by the negative control. The percentage of cell expressing the cell surface FcεRI is shown in each panel, and data are mean fluorescence intensity of CRA-1. The results shown are representative of three independent experiments.

tion was terminated by addition of 3 N HCl. The fluorescence intensity was measured with excitation at 360 nm and emission at 450 nm.

Statistical analysis

The statistical differences between control and *H. cordata* extract groups were determined by Student's *t* test. Differences were considered significant at $P < .05$.

RESULTS

Viability of KU812F cells

The viabilities of cells cultured in serum-free RPMI-1640 medium with various concentrations of *H. cordata* extract are given in Figure 1. *H. cordata* extract did not show any cytotoxic effects in the concentration range of 1–100 μg/mL.

Effects of *H. cordata* extract on cell surface expression of FcεRI

To evaluate *H. cordata* extract suppression of cell surface expression of FcεRI, KU812F cells were treated with different concentrations of *H. cordata* extract for 24 hours under serum-free conditions, and the cell surface expression of FcεRI was assessed by indirect immunofluorescence and flow cytometry using anti-FcεRI α-chain antibody, CRA-1.

The FcεRI expression on the cell surface was observed to be reduced from 33.4% to 31.4%, 24.0%, and 21.7% by treatment with *H. cordata* extract at 10, 50, and 100 μg/mL, respectively (Fig. 2).

Effects on FcεRI α-, β-, and γ-chain mRNA expression

To confirm *H. cordata* extract suppression of the gene expression of FcεRI, mRNA levels of FcεRI subunits were measured by RT-PCR using total cellular RNA prepared from KU812F cells treated with *H. cordata* extract. The mRNAs for the FcεRI α- and γ-chains of nontreated cells were clearly detected, and the level of the corresponding mRNA in the *H. cordata* extract-treated cells was shown to be reduced (Fig. 3). On the other hand, the β-chain mRNA was not detected in any of the conditions.

Effects on binding of IgE to FcεRI

The binding of IgE to the cell surface FcεRI was assessed by flow cytometric analysis using both human IgE and mite allergen-specific IgE. In the preliminary study, the serum obtained from peripheral blood of an allergic patient was proven to have a strong reactivity against mite allergen (data not shown). As shown in Figure 4A, the binding activity was observed to be 33.2%, 30.2%, 24.9%, and 20.8% at *H. cordata* extract treatment levels of 0, 10, 50, and 100 μg/mL, respectively. In addition, the binding activity of mite allergen-specific IgE to cell surface FcεRI was shown to be 20.1%, 13.0%, 11.7%, and 9.2% at the same level of *H. cordata* extract treatment (Fig. 4B).

Effects on FcεRI-mediated histamine release

To assess the inhibitory effect of *H. cordata* extract on the release of histamine, the level of histamine released from the cells by cross-linking of FcεRI with anti-FcεRI α-chain

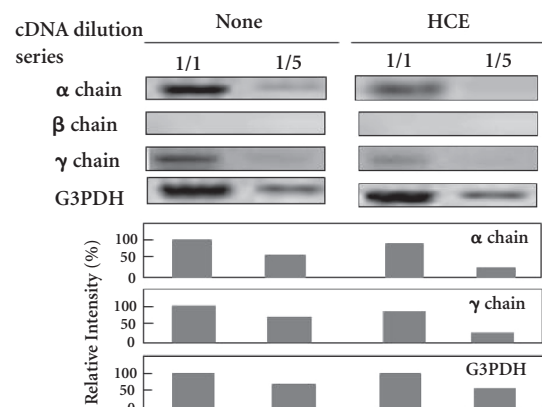


FIG. 3. Effects of *H. cordata* (HCE) on FcεRI α-, β-, and γ-chain mRNA expression. KU812F cells were cultured in 100 μg/mL HCE for 24 hours under serum-free conditions, total cellular RNA was isolated from the cells, and FcεRI α-, β-, and γ-chain and G3PDH mRNA levels were analyzed by RT-PCR.

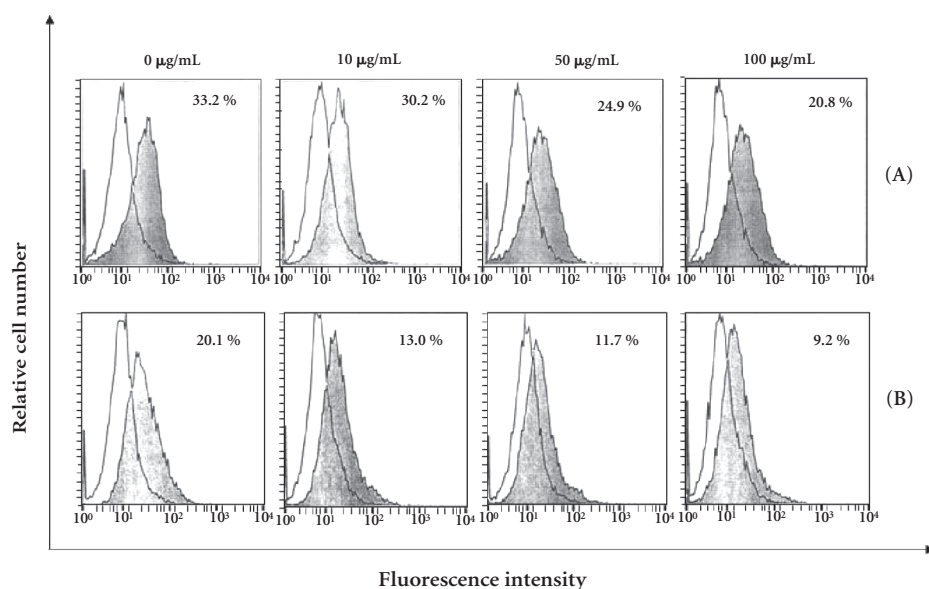


FIG. 4. Effects of *H. cordata* extract on binding of IgE antibody to cell surface FcεRI: (A) human myeloma IgE and (B) mite allergen-specific serum IgE. KU812F cells were cultured in the presence of different concentrations of *H. cordata* extract (0, 10, 50, or 100 μg/mL) for 24 hours. Then cells were incubated with human IgE or mite allergen-specific serum IgE antibodies followed by staining with FITC-conjugated IgE. Stained cells were then analyzed by flow cytometry. The percentage of positive cells was calculated with the arbitrary 2% cutoff position as determined by the negative control. The percentage of cell expressing the cell surface FcεRI is shown in each panel, and data are mean fluorescence intensity of IgE antibody. The results are representative of three independent experiments.

antibody, CRA-1, was measured. As shown in Figure 5, the FcεRI-mediated histamine release was 20.48 ± 0.58 ng/10⁶ cells in nontreated control cells. In *H. cordata* extract-treated cells, the release of histamine was $17.19 \pm 0.21\%$, $14.14 \pm 0.36\%$, and 10.63 ± 0.51 ng/10⁶ cells at *H. cordata* extract treatment levels of 10, 50, and 100 μg/mL, respectively.

DISCUSSION

Allergic diseases have rapidly increased in many industrialized countries and are becoming a major health problem throughout the world. In the present study, as part of a search for biologically active anti-allergic medicinal resources, the down-regulation of FcεRI expression by *H. cordata* was evaluated in human basophilic KU812F cells.

H. cordata has been used as a therapeutic drug in traditional Oriental medicine for treatment of allergy. However, experimental verifications of the effectiveness and the mechanism of action remain to be elucidated. Studies have reported that *H. cordata* inhibited anaphylactic shock caused by an injection of Compound 48/80.¹⁰ *H. cordata* also reduced histamine release in rat peritoneal mast cells in response to Compound 48/80 and substance P, and it decreased IgE production in spleen cells and human myeloma U266B1 cells.^{8–10} However, the effect of *H. cordata* on the regulation of FcεRI in allergic reaction has not been well characterized.

FcεRI is a high-affinity IgE receptor expressed on the cell surface of mast cells and basophils. It plays a key role in allergies by triggering IgE-mediated allergic reac-

tions.^{21–23} KU812F cells are a human basophilic cell line expressing the cell surface FcεRI. Expression of the cell surface FcεRI was suppressed in KU812F cells by the *H. cordata* extracts (Fig. 2). The suppression of cell surface FcεRI expression in the presence of *H. cordata* was found to be due to a decrease in the amount of total cellular FcεRI subunit gene expression (Fig. 3). FcεRI on basophils is a

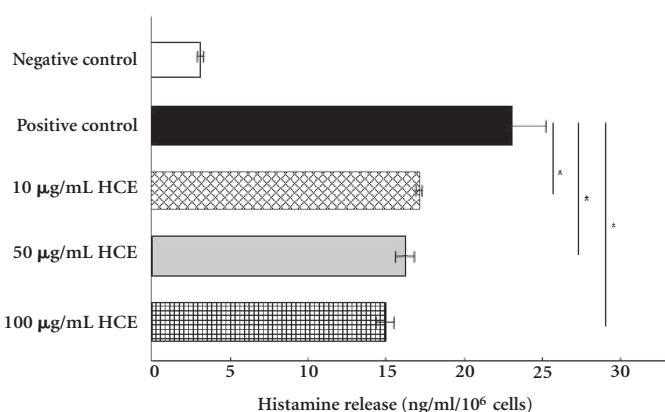


FIG. 5. Effects of *H. cordata* extract (HCE) on FcεRI-mediated histamine release. KU812F cells were cultured in the presence of different concentrations of HCE (0, 10, 50, or 100 μg/mL) for 24 hours. The cells were stimulated with CRA-1 antibody for 30 minutes. Histamine content was determined by a spectrofluorometric method. Data are mean $\pm$ SD values of three different experiments. *Values are significantly different from control ($P < .01$).

tetrameric molecule composed of one α -, one β -, and two γ -chains. The α -chain is expressed in Fc ϵ RI-positive cells and is essential for proper functioning of the cell surface receptor for IgE.^{16,24} Gene expression of the Fc ϵ RI subunits, as determined by the mRNA level with RT-PCR, was found to be down-regulated by *H. cordata* (Fig. 3). The gene expression of Fc ϵ RI α -chain is known to be regulated by at least two transcription factors, GATA and Elf-1, in rodents and mammals including humans.^{24–28} Further studies on the regulation of the signal transcription of the gene-encoding Fc ϵ RI α -chain are necessary for a better understanding of the suppressive mechanism of Fc ϵ RI expression by *H. cordata*.

Basophils and mast cells are sensitized by binding of the IgE molecule with their cell surface Fc ϵ RI and are degranulated by cross-linking of the cell surface Fc ϵ RI with IgE molecules and allergens.^{13,15} The binding of IgE antibody to cell surface Fc ϵ RI was found to be down-regulated by *H. cordata* in a concentration-dependent manner (Fig. 4A).

A number of indoor allergens have been known to be causes of perennial allergic diseases. House dust mites belonging to *Dermatophagoides* spp. are one of the major sources of allergens for allergic diseases, including atopic dermatitis.^{29,30} In the present study, binding of mite allergen-specific human serum IgE to cell surface Fc ϵ RI was found to be suppressed by *H. cordata* extract (Fig. 4B), indicating that *H. cordata* may reduce the risk of development and persistence of mite-derived allergic diseases.

Degranulation of cells through binding of IgE to cell surface Fc ϵ RI leads to secretion of inflammatory mediators such as histamine, which cause the symptoms of allergic inflammatory reactions.¹³ The release of histamine from KU812F cells mediated by Fc ϵ RI was determined by spectrofluorometric assay using *o*-phthalaldehyde and found to be reduced by *H. cordata* extract (Fig. 5). Considering the role of Fc ϵ RI in IgE-mediated allergic reaction, inhibition of allergen-IgE–Fc ϵ RI complex formation by *H. cordata* extract should also be useful to prevent allergic diseases. The results suggest that *H. cordata* extract might negatively regulate the activation of basophils through the suppression of Fc ϵ RI expression and thereby contribute to the attenuation of allergic reactions.

H. cordata contains flavonoids such as rutin, hyperoside, quercitrin, and quercetin with various biological and pharmaceutical functions.^{5,31} However, the inhibitory effects of Fc ϵ RI expression by *H. cordata* extract in basophils and mast cells have not been studied. Our findings provide the first experimental evidence that *H. cordata* inhibits Fc ϵ RI expression in human basophilic KU812F cells and that the suppressive effect of *H. cordata* extract on the cell surface expression of Fc ϵ RI is associated with the down-regulation of the mRNA expression of α - and γ -chains of Fc ϵ RI. The results suggest that *H. cordata* may play a role in the prevention and treatment of IgE-mediated allergic disease. Further studies on biologically active components of *H. cordata* are necessary to elucidate the suppressive mechanism of Fc ϵ RI expression.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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