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Antiallergic Tea Catechin, (−)-Epigallocatechin-3-*O*-(3-*O*-methyl)-gallate, Suppresses FcεRI Expression in Human Basophilic KU812 Cells

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We previously found that the *O*-methylated derivative of (−)-epigallocatechin-3-*O*-gallate (EGCg), (−)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG″3Me), has potent antiallergic activity. The high-affinity IgE receptor, FcεRI, is found at high levels on basophils and mast cells and plays a key role in a series of acute and chronic human allergic reactions. To understand the mechanism of action for the antiallergic EGCG″3Me, the effect of EGCG″3Me on the cell surface expression of FcεRI in human basophilic KU812 cells was examined. Flow cytometric analysis showed that EGCG″3Me was able to decrease the cell surface expression of FcεRI. Moreover, immunoblot analysis revealed that total cellular expression of the FcεRI α chain decreased upon treatment with EGCG″3Me. FcεRI is a tetrameric structure comprising one α chain, one β chain, and two γ chains. The level of mRNA production of each subunit in KU812 cells was investigated. EGCG″3Me reduced FcεRI α and γ mRNA levels. The cross-linkage of FcεRI causes the activation of basophils, which leads to the secretion of inflammatory mediators including histamine. EGCG″3Me treatment inhibited the FcεRI cross-linking-induced histamine release. These results suggested that EGCG″3Me can negatively regulate basophil activation through the suppression of FcεRI expression.

KEYWORDS: Tea catechin; basophil; KU812; FcεRI; *O*-methylated EGCg

INTRODUCTION

The high-affinity IgE receptor, FcεRI, plays a key role in a series of acute and chronic human allergic reactions such as atopic dermatitis, bronchial asthma, allergic rhitis, and food allergy (*1*, *2*). In humans and rodents, this receptor is found at high levels on basophils and mast cells where activation by cross-linking of the allergen-specific IgE bound to FcεRI with multivalent allergens produces several mediators including histamine, proteases, chemotactic factors, and arachidonic acid metabolites responsible for FcεRI-dependent allergic reactions. IgE-binding and signal-transducing functions for the FcεRI molecule are performed separately by distinct subunits (*1*, *3*). FcεRI is a tetrameric receptor consisting of one α chain, one β chain, and two disulfide-linked γ chains. In humans, the tetrameric structure is not obligatory. An alternative form is present comprising an $\alpha\gamma_2$ trimer. The FcεRI α chain mostly extends out to the extracellular region of the FcεRI and binds to the Fc portion of the IgE with high affinity. Studies on the FcεRI α chain knockout mice demonstrated that IgE cannot bind to the cell surface of mast cells, thereby degranulation through IgE-binding was not induced (*4*). Thus, it is expected that the down-regulation of FcεRI expression in mast cells and basophils leads to the attenuation of the IgE-mediated allergic symptoms. However, evaluations of anti- or proallergic factors in foodstuffs so far performed are based almost entirely on the chemical mediator release test (*5*−*7*). Our previous report demonstrated that an evaluation of antiallergic activity in foodstuffs can be based on the suppression of FcεRI expression (*8*).

Tea (*Camellia sinensis* L.) is one of the most widely consumed beverages in the world, and it is known to contain various beneficial constituents. It has been demonstrated that these tea constituents exhibit various biological and pharmacological properties, and have been reported to act in several ways that are antioxidative (*9*, *10*), and antimutagenic or anticarcinogenic (*11*, *12*). Catechins apparently have an essential role in these actions, and (−)-epigallpcatechin-3-*O*-gallate (EGCg), which is the major catechin in tea leaves, is believed to be most responsible for their beneficial effects. Recently, the *O*-methylated derivative of EGCg, (−)-epigallpcatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG″3Me), which was isolated from Tong ting oolong tea and Benihomare tea leaves (*Camellia*

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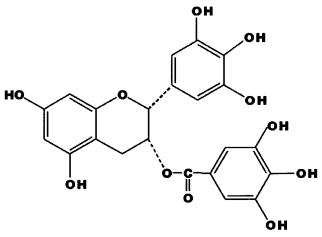
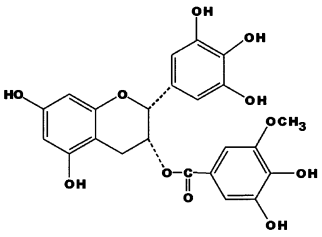


(-)-Epigallocatechin-3-*O*-gallate (EGCg)



(-)-Epigallocatechin-3-*O*-(3-*O*-methyl) -gallate (EGCG"3Me)

Figure 1. Chemical structures of EGCg and the *O*-methylated derivative.

sinensis L.), has been shown to inhibit type I allergy more potently than EGCg. We also found that EGCG″3Me can inhibit histamine release in the human basophilic cell line KU812 (*13*). In the present study, we investigated whether antiallergic catechin EGCG″3Me has a suppressive effect on FcεRI expression in KU812 cells.

MATERIALS AND METHODS

Reagents. Protein A Sepharose beads was purchased from Amersham Pharmacia Biotech. Mouse anti-human FcεRI α chain monoclonal antibody CRA-1 was obtained from Kyokuto Seiyaku (Tokyo, Japan). Mouse IgG2b antibody and fluorescein isothiocyanate (FITC)-conjugated mouse IgG1 antibody used for negative control were bought from Dako. (−)-Epigallocatechin-3-*O*-gallate (EGCg) was purchased from Kurita Water Industries LTD (Tokyo, Japan). (−)-Epigallocatechin-3-*O*-(3-*O*-methyl) gallate was prepared from Benihomare tea leaves (*Camellia sinensis* L. cv. benihomare) according to the method described previously (*15*). The chemical structures of these catechins are shown in **Figure 1**.

Cell Culture and Stimulation. KU812 cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan) and were maintained in RPMI-1640 (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY), 100 U/mL penicillin G, 100 mg/mL streptomycin, and 10 mM HEPES buffer. KU812 cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. Tea catechins, EGCg and EGCG″3Me, were dissolved in distilled water. For stimulation with these tea catechins, KU812 cells were first centrifuged and washed with RPMI1640 medium. Then the cells were cultured in serum-free RPMI-1640 medium with or without tea catechins.

Flow Cytometric Analysis. The cell surface expression of FcεRI was assessed by flow cytometry. In brief, cells were incubated with the anti-FcεRI α chain antibody CRA-1 for 60 min at 4 °C. Then the cells were washed twice in phosphate-buffered saline (PBS; pH 7.5) and exposed to the FITC-conjugated $F(ab')_2$ goat anti-mouse IgG for 60 min at 4 °C. The cells were washed twice in PBS and subjected to flow cytometry (FACSCalibur; Becton Dickinson, Sunnyvale, CA). As a negative control, mouse subclass-matched polyclonal IgG2b antibody was used. The extent of FcεRI expression is represented as the mean fluorescence intensity of CRA-1.

Immunoprecipitation and Immunoblot Analysis. KU812 cells were cultured with 50 μM catechin for 24 h under serum-free conditions, then 2×10^7 cells were lysed in cell lysis buffer containing 0.01 M Tris−HCl, pH 8.0, 0.14 M NaCl, 10% Triton X-100, 0.025% NaN_3, 1.0 mM phenylmethanesulfonyl fluoride (PMSF), and 2.0 μg/mL aprotinin. Whole cell lysates were incubated with protein A Sepharose beads overnight at 4 °C. After centrifugation, cell lysates were incubated with protein A Sepharose beads bound with the anti-FcεRI α chain antibody CRA-1 for 4 h at 4 °C. The beads were washed 3 times with lysis buffer, and then resuspended in sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) buffer containing 2% 2-mercaptoethanol, and boiled for 5 min. The beads were removed by centrifugation, and the supernatants were subjected to Western blot analysis. The immunoprecipitates were loaded onto an 8% SDS−PAGE gel, and electrophoresis was done under reducing conditions. The samples were then electrotransferred onto a nitrocellulose membrane. The blotted nitrocellulose was probed for FcεRI α chain using the anti-FcεRI α chain antibody CRA-1. The secondary antibody was horseradish peroxidase (HRP)-conjugated anti-mouse IgG, and detection was done using the ECL kit (Amersham Pharmacia Biotech).

Analysis of FcεRI α, *β*, and *γ* mRNA Expression. Total cellular RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. For cDNA synthesis, 10 μg of total RNA was reversely transcribed using a $(dT)_{20}$ primer and 20 U of Moloney mouse leukemia virus (MMLV)-reverse transcriptase (Amersham Pharmacia Biotech). The resultant cDNA samples were subjected to PCR amplification in the presence of specific sense and antisense primers. Sequences for the PCR primers were as follows: for the FcεRI α chain, sense 5′-CTTAGGATGTGGGTTCAGAAGT-3′, and antisense 5′-GACAGTGGAGAATACAAATGTCA-3′; for the FcεRI *β* chain, sense 5′-TAATTCTTCATAAAGACGATCATC (A, G, C, or T) GG-3′, and antisense 5′-ATATGCCTTTGTTTTGGAA-CAATGGTGTG-3′; for the FcεRI *γ* chain, sense 5′-TAGGGCCAGC-TGGTGTTAATGGCA-3′, and antisense 5′-GATGATTCCAGCAGTG-GTCTTGCT-3′; for G3PDH, sense 5′-GCTCAGACACCATGGG-GAAGGT-3′, and antisense 5′-GTGGTGCAGGAGGCATTGCTGA-3′. The amplified PCR products were subjected to electrophoresis on a 1.5% agarose gel, and transferred to a Hybond-N^+ membrane (Amersham Pharmacia Biotech) and hybridized with probes specific for the FcεRI α, *β*, and *γ* chains and G3PDH, respectively. The hybridized probe was detected using the Gene Images detection kit (Amersham Pharmacia Biotech).

Histamine Release Assay. KU812 cells (1.0×10^6 cells/mL) were pretreated with 50 μM catechin for 24 h. The treated cells were washed and suspended in 200 μL of histamine release buffer (pH 7.2), containing 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, and 11.9 mM NaH_2PO_4, and then stimulated with 20 μg/mL CRA-1 at 37 °C for 30 min. After centrifugation at 300*g* for 5 min, the histamine content of the supernatant or cell lysate was measured by means of a fluorometric assay.

RESULTS

Effect of EGCG″3Me on the Cell Surface Expression of FcεRI in KU812 Cells. EGCG″3Me was separated from Benihomare cultivar, which is one of the cultivars used for Japanese black tea. We previously reported that EGCG″3Me inhibited degranulation from human basophilic KU812 cells more effectively than EGCg (*13*). The chemical structure of EGCG″3Me is shown in **Figure 1**, and this catechin is the *O*-methylated form of the galloyl moiety in EGCg. In this study, the effect of EGCG″3Me on the expression of FcεRI on the cell surface of KU812 cells was investigated. The cells were treated with EGCG″3Me at a concentration of 0, 1, 5, 10, 25, or 50 μM for 24 h under serum-free conditions. Because the expression of the FcεRI α chain is limited to FcεRI-expressing cells and the FcεRI α chain mostly extends out to the extracellular region of the FcεRI, the cell surface expression of FcεRI was measured by flow cytometric analysis using the anti-FcεRI α chain antibody CRA-1. As shown in **Figure 2A**, the suppressive effect of EGCG″3Me on FcεRI expression was

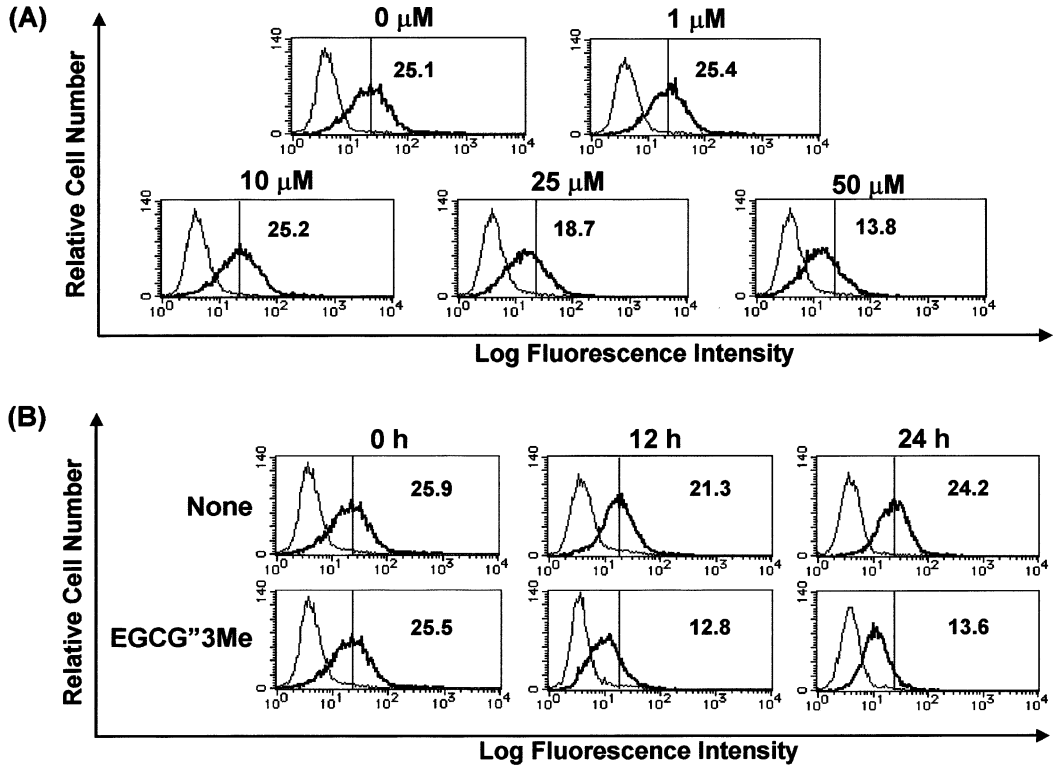


Figure 2. Effect of EGCG″3Me on the cell surface expression of FcεRI. (A) KU812 cells were cultured in the presence of different concentrations of EGCG″3Me (0, 1, 10, 25, and 50 μM) for 24 h. (B) KU812 cells were cultured with or without 50 μM EGCg or EGCG″3Me for 0, 12, or 24 h. Then, cells were incubated with CRA-1 (solid line) followed by staining with the FITC-conjugated goat anti-mouse IgG. Mouse IgG2b (thin line) was used as the isotype-matched negative control. The value indicated in the figure is the mean fluorescence intensity of CRA-1. The vertical line in the figure indicates the peak point for CRA-1 in the nontreated cells. Three independent trials were performed, and the histograms shown are from a representative experiment.

demonstrated in the presence of more than 25 μM. At time point 0 h, treatment with EGCG″3Me did not suppress the level of FcεRI expression (**Figure 2B**). The suppression of FcεRI expression was detected at 12 h after EGCG″3Me addition and was sustained for a 24-h period. These results suggest that the suppression of FcεRI expression by EGCG″3Me requires at least a 12-h stimulation.

Effect of EGCG″3Me on the Level of Total Cellular FcεRI α Chain Protein. To examine whether this EGCG″3Me-mediated suppression of FcεRI cell surface expression is due to a decrease in the amount of total cellular FcεRI α chain produced, we performed an immunoblot analysis and compared EGCG″3Me with EGCg. After a 50 μM EGCG″3Me or EGCg treatment for 24 h, the cells were lysed and immunoprecipitated with CRA-1, followed by immunoblot analysis. As shown in **Figure 3**, the level of total cellular FcεRI α chain decreased upon treatment with EGCG″3Me, which was slightly lower than that with EGCg. This result indicates that the EGCG″3Me-induced decrease in the level of cellular FcεRI α chain may be associated with the suppression of FcεRI cell surface expression.

Effect of EGCG″3Me on FcεRI α, *β*, and *γ* mRNA Expression. We also examined whether the suppressive effect of EGCG″3Me on the FcεRI α chain expression is due to the inhibition of mRNA expression for the FcεRI α chain gene. KU812 cells were cultured for 24 h with or without 50 μM of EGCG″3Me or EGCg, and total RNA was isolated from the cells. The mRNA levels for the α chain as well as the *β* and *γ* chains were measured by RT-PCR followed by Southern blotting (**Figure 4**). The FcεRI α and *γ* mRNA of nontreated cells were clearly detected, and the corresponding mRNA levels in the EGCG″3Me-stimulated cells were shown to be significantly

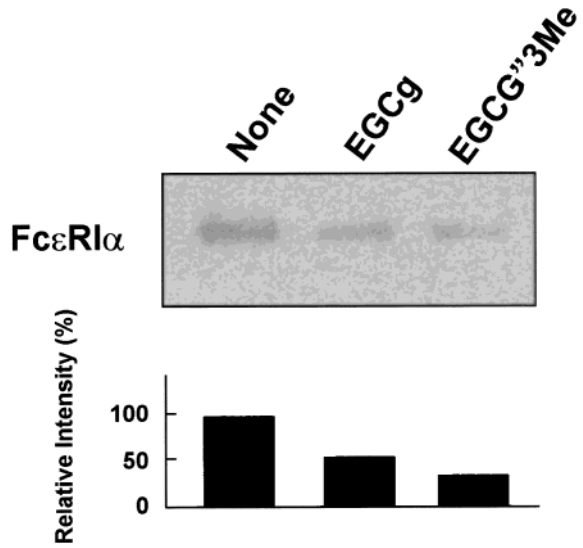


Figure 3. Immunoblot analysis of the cellular FcεRI α chain protein expression in KU812 cells treated with catechins. Cells were treated with 50 μM catechins under serum-free conditions for 24 h. Immunoblot analysis was performed on whole cell lysates using the anti-FcεRI α chain antibody CRA-1.

reduced. EGCG″3Me was able to suppress the FcεRI α chain more than EGCg. The FcεRI *β* mRNA was not detected in any of the three conditions. Thus, it was suggested that the suppressive effect of EGCG″3Me on the cell surface expression of FcεRI is at least related to the down-regulation of the expression of the FcεRI α and *γ* mRNA.

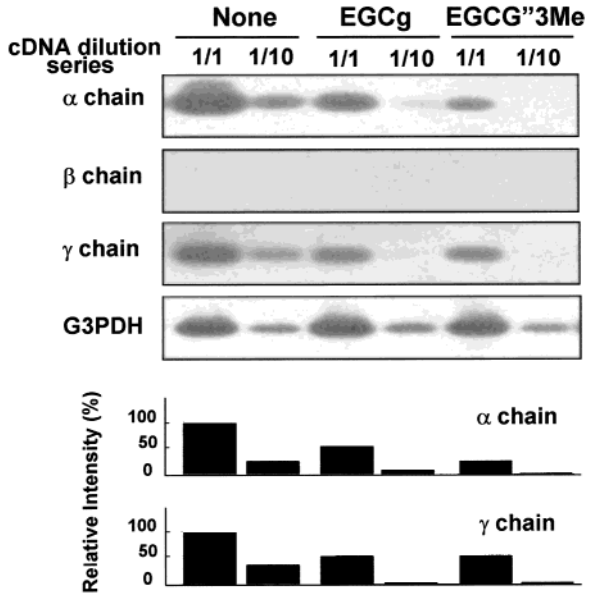


Figure 4. Analysis of the mRNA level for FcεRI α, β, and γ chains in KU812 cells treated with catechins. After treatment with 50 μM catechins for 24 h under serum-free conditions, total RNA was isolated from the cells. FcεRI α, β, and γ and G3PDH mRNA were analyzed by RT-PCR. Southern blotting using specific probes for FcεRI α, β, and γ and G3PDH was performed to assess the PCR products.

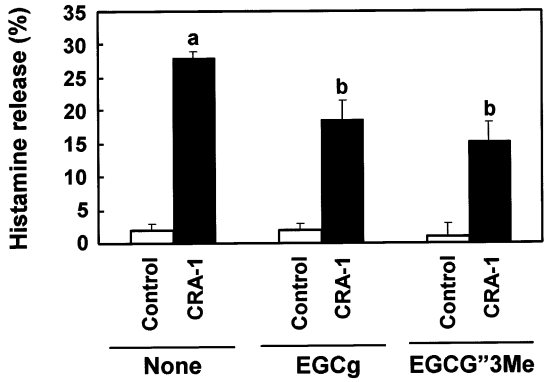


Figure 5. Inhibitory effect of catechins on histamine release from KU812 cells stimulated with anti-FcεRI mAb (CRA-1). Cells (1×10^6 cells/mL) treated with catechins for 24 h were stimulated with 20 μg/mL CRA-1 at 37 °C for 30 min. Control indicated in the figure was used as the isotype-matched negative control. Histamine content was determined using a fluorometric assay. Each data value is expressed as mean ± SD ($n = 3$). Values not sharing a common letter (a, b) are significantly different between groups ($p < 0.05$).

Inhibitory Effect of EGCG″3Me on FcεRI-Mediated Histamine Release from KU812 Cells. To assess whether the reduction of FcεRI expression by EGCG″3Me is associated with a functional change, especially histamine release, we cultured KU812 cells for 24 h with or without either EGCG″3Me or EGCg, and then stimulated the cells with the FcεRI specific antibody CRA-1. Histamine released from the cells was determined using a fluorometric assay (**Figure 5**). In nontreated cells with the catechins, the value of FcεRI-mediated histamine release was 27.8 ± 1.2 ng/10^6 cells. After treatment with EGCg or EGCG″3Me, 18.6 ± 2.9 or 15.2 ± 3.8 ng/10^6 cells of histamine release were observed, respectively. This observation indicated that the reduction of histamine release was probably caused by the suppression of FcεRI expression by EGCg and EGCG″3Me.

DISCUSSION

We showed that the antiallergic catechin EGCG″3Me reduced the expression of FcεRI α and γ chain genes. With regard to the mechanism for FcεRI expression, it has recently been described that the α chain expression is regulated by two transcription factors, GATA-1 and Elf-1, in rodents and other mammals such as humans (*15*). The expression of FcεRI has been shown to be up-regulated by interleukin-4 (*16*, *17*), which is known to transfer a signal to the signal transducers and activators of transcription (STAT) molecule STAT6 motif. As for the γ chain, it is recognized as an essential molecule for enabling the FcεRI α chain to be expressed on the cell surface, and plays a critical role in signal transduction (*1*, *2*). Unlike the expression of α chain, which is limited to the cells expressing FcεRI, the γ chain is known to be a common component of other Fc receptors and T cell receptor complexes, but its regulatory mechanism is not fully understood (*2*, *18*, *19*). The FcεRI β chain, as shown in **Figure 4**, was not expressed in KU812 cells. In human cells, the $\alpha\gamma_2$ trimer has been previously demonstrated as an alternative form of FcεRI, and the β chain has been shown to be unnecessary for the expression of functional human FcεRI on the cell surface (*20*). Therefore, the β chain may not be involved in the suppression of FcεRI expression by EGCG″3Me. To better understand the suppressive mechanism of FcεRI expression, especially the down-regulation of the α and γ chains by EGCG″3Me, further studies on molecular mechanism, such as the involvement of several transcription factors, are necessary.

We previously demonstrated that EGCg can suppress FcεRI expression, and this suppressive effect may be caused by down-regulating the protein or mRNA expression of the α chain as well as that of the γ chain (*8*). Here, EGCG″3Me is shown to be able to suppress their expressions similar to EGCg. Moreover, it was observed that EGCG″3Me reduced the α chain expression at protein or mRNA levels more strongly than EGCg. In addition, the inhibition of histamine release from KU812 cells treated with EGCG″3Me in response to cross-linkage of FcεRI was also higher than that of EGCg. Previously, we indicated that methylation of EGCg may be effective in inhibiting degranulation from human basophils (*13*). These results support the belief that methylation of catechins may enhance their antiallergic activities.

It is generally known that catechins are absorbed and are present as methylated, glucuronidated, and sulfated conjugates in human plasma (*21*−*23*). Not only the intact form of catechins, but also the catechin-derived metabolites which have undergone *O*-methylation, glucuronidation, and sulfation may interact with target molecules to exert a physiological activity. Recently, the effects of *O*-methylated derivatives of EGCg have been reported (*14*, *24*). The inhibitory effects of the EGCg *O*-methylated derivatives on mouse type I and IV allergies were stronger than those of EGCg. It has been speculated that the higher antiallergic activity of the EGCg *O*-methylated forms might be associated with stability in vivo (*14*, *24*). It has also been shown that EGCG″3Me is more stable than EGCg in mouse plasma in an in vitro study, and EGCG″3Me is produced when EGCg is incubated with mouse liver homogenate or an authentic catechol-*O*-methyltransferase (*14*), which catalyzes *O*-methylation of various plant polyphenols (*25*). Thus, EGCg in vivo may also be partly converted to EGCG″3Me by the methyltransferase, and these catechin complexes may have antiallergic properties. Our current report showed that EGCg has the ability not only to inhibit degranulation but also to decrease FcεRI expression in human basophils, and that EGCG″3Me has an inhibitory

effect on degranulation (*8*, *13*). These observations and present findings may provide a suggestion for antiallergic effects of EGCg and its derivatives in vivo.

Both the EGCg and EGCG″3Me-induced modulation of FcεRI expression may be of particular importance, because such effects can theoretically influence all FcεRI-mediated downstream events. For example, dexamethasone-treated mouse mast cells have shown to be a reduced degranulation response to FcεRI cross-linkage through down-regulation of surface FcεRI expression (*26*). Here, we demonstrated that modulation of FcεRI expression by EGCg and EGCG″3Me leads to inhibition of histamine release. Cross-linking of FcεRI on the cell membrane induces elevation of intracellular Ca^{2+} concentration, which leads to the secretion of inflammatory mediators such as histamine. In a previous study, we described that EGCg and EGCG″3Me inhibit the calcium ionophore A23187-induced histamine release, and speculated that these inhibitory effects may be exerted by down-regulating the signal events occurring after Ca^{2+} influx (*13*). However, the reduction of FcεRI-stimulated histamine release shown here may be a result from a decrease in FcεRI expression initiated by culturing with EGCg and EGCG″3Me. So far, it has been reported that FcεRI is expressed not only on mast cells and basophils, but also on dermal Langerhans cells, monocytes, eosinophils, dendritic cells, and platelets (*27*, *28*−*30*). It is thought that FcεRI may promote allergic inflammation via action including release of chemical mediators, antigen presentation, and adhesion on a range of hematopoietic cells in addition to basophils and mast cells (*31*). Thus, the decrease of FcεRI expression by antiallergic tea catechins such as EGCg and EGCG″3Me raises the possibility that these compounds might negatively regulate the activation of basophils and mast cells as well as other hematopoietic cells and contribute to attenuattion of allergic reactions.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Blank, U.; Ra, C.; Miller, L.; White, K.; Metzer, H.; Kinet, J.-P. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature* **1989**, *337*, 187−189.

(2) Blank, U.; Ra, C.; Kinet, J.-P. Characterization of the truncated α chain products from human, Rat and mouse high affinity receptor for Immunoglobulin E. *J. Biol. Chem.* **1991**, *266*, 2639−2646.

(3) Ravetch, J. V.; Kinet, J.-P. Fc receptors. *Annu. Rev. Immunol.* **1991**, *9*, 457−492.

(4) Dombrowicz, D.; Flamand, V.; Brigman, K.; Koller, BH.; Kinet, J.-P. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* **1993**, *75*, 969−976.

(5) Ohmori, Y.; Ito, M.; Kishi, M.; Mizutani, H.; Katada, T.; Konoshi, H. Antiallergic constituents from oolong tea stem. *Biol. Pharm. Bull.* **1995**, *18*, 683−686.

(6) Matsuo, N.; Yamada, K.; Shoji, K.; Sugano, M. Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-inhibitory activity relationship. *Allergy* **1997**, *52*, 58−64.

(7) Yamashita, K.; Suzuki, Y.; Matsui, T.; Yoshimaru, T.; Yamaki, M.; Suzuki-Karasaki, M.; Hayakawa, S.; Shimizu, K. Epigallocatechin gallate inhibit histamine release from rat basophilic leukemia (RBL-2H3) cells: Role of tyrosine phosphorylation pathway. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 603−608.

(8) Fujimura, Y.; Tachibana, H.; Yamada, K. A tea catechin suppresses the expression of the high affinity IgE receptor FcεRI in the human basophilic KU812 cells. *J. Agric. Food Chem.* **2001**, *49*, 2527−2531.

(9) Yoshino, K.; Hara, Y.; Sano, M.; Tomita, I. Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by *tert*-butyl hydroperoxide. *Biol. Pharm. Bull.* **1994**, *17*, 146−149.

(10) Sano, M.; Takahashi, Y.; Yoshino, K.; Shimoi, K.; Nakamura, Y.; Tomita, I.; Oguni, I.; Konomoto, H. Effect of tea (*Camellia sinensis* L.) on lipid peroxidation in rat liver and kidney: a comparison of green and black tea feeding. *Biol. Pharm. Bull.* **1995**, *18*, 1006−1008.

(11) Yamane, T.; Nakatani, H.; Kikuoka, N.; Matsumoto, H.; Iwata, Y.; Kitao, Y.; Oya, K.; Takahashi, T. Inhibitory effects and toxicity of green tea polyphenols for gastrointestinal carcinogenesis. *Cancer* **1996**, *77* (8 Suppl), 1662−1667.

(12) Yen, G. C.; Chen, H. Y. Relationship between antimutagenic activity and major components of various teas. *Mutagenesis* **1996**, *11*, 37−41.

(13) Tachibana, H.; Sunada, Y.; Miyase, T.; Sano, M.; Yamamoto-Maeda, M.; Yamada, K. Identification of a methylated tea catechin as inhibitor of degranulation in human basophilic KU812 cells. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 452−454.

(14) Sano, M.; Suzuki, M.; Miyase, T.; Yoshino, K.; Maeda-Yamamoto, M. Novel antiallergic catechin derivatives isolated from oolong tea. *J. Agric. Food Chem.* **1999**, *47*, 1906−1910.

(15) Nishiyama, C.; Yokota, T.; Okumura, K.; Ra, C. The transcription Factors Elf-1 and GATA-1 bind to cell-specific enhancer elements of human high-affinity IgE receptor α-chain gene. *J. Immunol.* **1999**, *163*, 623−630.

(16) Toru, H.; Ra, C.; Nonoyama, S.; Suzuki, K.; Yata, J. I.; Nakata, T. Induction of the high-affinity IgE receptor (FcεRI) on human mast cells by IL-4. *Int. Immunol.* **1996**, *8*, 1367−1373.

(17) Hara, T.; Yamada, K.; Tachibana, H. Basophilic differentiation of the human leukemia cell line KU812 upon treatment with interleukin-4. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 542−548.

(18) Letourneur, F.; Klausner, R. D. T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor ζ family proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8905−8909.

(19) Orloff, D. G.; Ra, C.; Frank, S. J.; Klausner, R. D.; Kinet, J.-P. Family of disulfide-linked dimers containing the ζ and η chains of the T-cell receptor and γ chain of Fc receptors. *Nature* **1990**, *347*, 189−191.

(20) Miller, L.; Blank, U.; Metzger, H.; Kinet, J.-P. Expression of high affinity binding of human immunoglobulin E by transfected cells. *Science* **1989**, *244*, 334−337.

(21) Hackett, A. M.; Griffiths, L. A.; Broillet, A.; Wermeille, M. The metabolism and excretion of (+)-[14C]cyanidanol-3 in man following oral administration. *Xenobiotica* **1983**, *13*, 279−286.

(22) Lee, M. J.; Wang, Z. Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, S.; Balentine, D. A.; Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomark. Prev.* **1995**, *4*, 393−399.

(23) Donovan, J. L.; Bell, J. R.; Kasim-Karakas, S.; German, J. B.; Walzem, R. L.; Hansen, R. J.; Waterhouse, A. L. Catechin is present as metabolites in human plasma after consumption of red wine. *J. Nutr.* **1999**, *129*, 1662−1668.

(24) Suzuki, M.; Yoshino, K.; Maeda-Yamamoto, M.; Miyase, T.; Sano, M. Inhibitory effects of tea catechins and O-methylated derivatives of (−)-epigallocatechin-3-*O*-gallate on mouse type IV allergy. *J. Agric. Food. Chem.* **2000**, *48*, 5649−5653.

(25) Guldberg, H. C.; Marsden, C. A. Catechol-*O*-methyl transferase: pharmacological aspects and physiological role. *Pharmacol. Rev.* **1975**, *27*, 135−206.

(26) Yamaguchi, M.; Hirai, K.; Komiya, A.; Miyamasu, M.; Furumoto, Y.; Teshima, R.; Ohta, K.; Galli, S. J.; Ra, C.; Yamamoto, K. Regulation of mouse mast cell surface Fc epsilon RI expression by dexamethasone. *Int. Immunol.* **2001**, *13*, 843−851.

(27) Wang, B.; Rieger, A.; Kilugus, O.; Ochiai, K.; Maurer, D.; Fodinger, D.; Kinet, J.-P.; Stingl, G. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. *J. Exp. Med*. **1992**, *175*, 1353−1365.

(28) Maurer, D.; Fiebiger, E.; Ebner, C.; Reininger, B.; Fischer, G. F.; Wichlas, S.; Jouvin, M. H.; Schmitt-Egenolf, M.; Kraft, D.; Kinet, J.-P.; Stingl, G. Peripheral blood dendritic cells express FcϵRI as a complex composed of FcϵRIα- and FcϵRIγ-chains and can use this receptor for IgE-mediated allergen presentation. *J. Immunol*. **1996**, *157*, 607−616.

(29) Gounni, A. S.; Lamkhioued, B.; Ochiai, K.; Tanaka, Y.; Delaporte, E.; Capron, A.; Kinet, J.-P.; Capron, M. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* **1994**, *367*, 183−186.

(30) Hasegawa, S.; Pawankar, R.; Suzuki, K.; Nakahata, T.; Furukawa, S.; Okumura, K.; Ra, C. Functional expression of the high affinity receptor for IgE (FcepsilonRI) in human platelets and its' intracellular expression in human megakaryocytes. *Blood* **1999**, *93*, 2543−2551.

(31) Oliver, J. M.; Kepley, C. L.; Ortega, E.; Wilson, B. S. Immunologically mediated signaling in basophils and mast cells: finding therapeutic targets for allergic diseases in the human FcϵRI signaling pathway. *Immunopharmacology* **2000**, *48*, 269−281.

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