JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Flavones Suppress the Expression of the High-Affinity IgE Receptor FcεRI in Human Basophilic KU812 Cells

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We examined the effect of flavones on the expression of the high-affinity IgE receptor FcεRI, which plays a central role in the IgE-mediated allergic response. Flow cytometric analysis showed that the flavones chrysin and apigenin were able to reduce the cell surface expression of FcεRI in human basophilic KU812 cells. Immunoblot analysis revealed that the total cellular expression of the FcεRI α and γ chains decreased upon treatment with chrysin and apigenin. Moreover, the level of mRNA expression of the FcεRI α and γ chains also decreased when the cells were cultured with the two flavones. Previously, we demonstrated that the reduction of extracellular signal-regulated kinase1/2 (ERK1/2) phosphorylation was involved in the downregulation of FcεRI expression. The two flavones were shown to reduce the level of ERK1/2 phosphorylation. These results suggested that chrysin and apigenin have the ability to downregulate FcεRI expression and this suppressive effect may be due to the reduction of ERK1/2 phosphorylation.

KEYWORDS: Chrysin; apigenin; basophil; KU812; FcεRI; ERK1/2

INTRODUCTION

Mast cells and basophils express FcεRI on their cell surface, and the cross-linking of the allergen-specific IgE bound to FcεRI leads to the release of inflammatory mediators. Therefore, FcεRI is a key molecule in triggering an IgE-mediated allergic reaction such as in atopic dermatitis, bronchial asthma, allergic rhitis, and food allergy (*1*, *2*). FcεRI is a tetrameric structure consisting of an α chain, a β chain, and two 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 and binds to the Fc portion of IgE with high affinity, and the γ chain transduces signals (*3*). The β chain is an amplifier of signal strength (*4*, *5*) and enhances the expression of unoccupied receptors on the cell surface (*6*). Analysis of FcεRI α chain-deficient mice demonstrated that IgE cannot bind to the cell surface of mast cells and that degranulation through IgE-binding cannot be induced (*7*). Thus, it is expected that the downregulation of FcεRI expression in mast cells and basophils can lead to the attenuation of the IgE-mediated allergic symptoms. However, evaluation of anti- or proallergic factors in foodstuffs so far performed is based almost entirely on the chemical mediator release inhibitory effect (*8*−*10*).

The flavonoids are a diverse family of chemicals commonly found in fruits and vegetables. Flavonoids are plant polyphenolic compounds, which have a diphenylpropane skeleton (C6C3C6) structure which include monomeric flavanols, flavanones, flavones, and flavonols. Some flavonoids possess various clinically relevant properties such as antitumor, antiplatelet, anti-ischemic, and anti-inflammatory activities (*11*, *12*). Moreover, some flavonoids were previously shown to inhibit histamine release from human basophils and rat mast cells (*13*, *14*).

Our previous report demonstrated that a tea catechin, (−)-epigallocatechin-3-*O*-gallate (EGCG), can suppress FcεRI expression (*15*). In the present study, we chose two flavones, chrysin and apigenin, to examine the inhibitory effect on FcεRI expression in the human basophilic cell line KU812.

MATERIALS AND METHODS

Reagents. Chrysin and apigenin were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich Chem. Co. (St. Louis, MO), respectively. Stock solutions of chrysin and apigenin were prepared in 10% dimethyl sulfoxide (DMSO) and ethanol. Protein A Sepharose beads were purchased from Amersham Biosciences Corp. (Piscataway, U.S.). Mouse antihuman FcεRI α chain monoclonal antibody CRA-1 was obtained from Kyokuto Seiyaku (Tokyo, Japan). Mouse IgG2b antibody, which was used as a negative control, was purchased from Dako Cytomaition (Denmark). Fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG antibody was purchased from Protos Immunoresearch (Burlingame, CA). Human anti-FcεRI γ subunit, TCR, Fc receptor rabbit polyclonal IgG was purchased from Upstate cell signaling solutions (Lake Placid, New York). Mouse antiphosphorylated ERK1/2 antibody and rabbit anti-ERK1/2 antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase (HRP)-conjugated antimouse IgG antibody and HRP-conjugated antirabbit antibody were obtained from Zymed Laboratories, Inc. (San Francisco, CA) and ICN Pharmaceuticals, Inc. (Aurora, OH), respectively. PD98059 was obtained from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA).

Cell Culture and Stimulation. KU812 cells were obtained from the Japanese Cancer Resources Bank (Tokyo, Japan) and were main-

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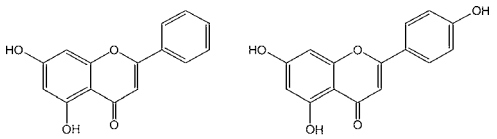
10.1021/jf047929d CCC: $30.25
Published on Web 02/09/2005

tained in RPMI 1640 (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY), 100 units/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. For stimulation with flavonoids, KU812 cells were centrifuged and washed with RPMI 1640 medium. Then, the cells were cultured in serum-free RPMI 1640 medium with or without flavones or PD98059.

Flow Cytometric Analysis of Cell Surface FcεRI Expression. The cell surface expression of FcεRI was assessed by flow cytometry. KU812 cells cultured in serum-free RPMI 1640 medium with or without flavones or PD98059 were incubated with the anti-FcεRI α chain mouse monoclonal antibody for 60 min at 4 °C. Then, the cells were washed once in phosphate-buffered saline (PBS) and exposed to the FITC-conjugated $F(ab')_2$ goat antimouse IgG for 60 min at 4 °C. The cells were again washed in PBS, and then detection of the cell surface FcεRI α chain was performed using the FACSCalibur flow cytometer (Becton Dickinson, Sunnyvale, CA). Mouse IgG2b antibody was used as the isotype-matched negative control.

Immunoprecipitation and Immunoblot Analysis. KU812 cells were cultured with 25 μM flavones for 24 h under serum-free conditions. Cells were rinsed once with PBS and lysed in 1% Triton X-100 lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 30 mM $Na_4P_2O_7$, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/mL aprotinin). Insoluble material was removed by centrifugation at 12 000*g* for 10 min at 4 °C. Prior to analysis, total protein in the cell lysates was measured using a colorimetric BCA protein assay (Pierce Biotechnology, Inc., U.S.) against bovine serum albumin standards. After preclearing with protein A Sepharose beads, anti-FcεRI α chain antibody was added for 4 h at 4 °C. Whole cell lysates were immunoprecipitated with anti-FcεRI α chain antibody prebounded to protein A Sepharose beads overnight at 4 °C. The beads were washed three times with lysis buffer and PBS, and then the bound proteins were eluted with sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) sample buffer. Immunoprecipitates or cell lysates were run on a 10 or 15% SDS−PAGE gel and blotted onto nitrocellulose membranes. After blocking, proteins were identified using anti-FcεRI α mouse monoclonal IgG and anti-FcεRI γ rabbit polyclonal IgG antibodies. The bands were visualized with HRP-conjugated antimouse IgG or antirabbit IgG antibodies, followed by visualization using the ECL system (Amersham Pharmacea Biotech, U.S.). For examination of ERK1/2, the cells were treated with 25 μM flavonoids for 0, 1, 3, and 6 h and then were rinsed once with PBS and lysed in 1% Triton X-100 lysis buffer added with 1 mM pervanadate. Insoluble material was removed by centrifugation at 12 000*g* for 10 min at 4 °C. The whole cell lysates were resuspended in SDS−PAGE sample buffer and subjected to 10% SDS−PAGE and immunoblotted using the antiphosphorylated ERK1/2 antibodies and the HRP-conjugated antirabbit IgG antibody. For detection of total ERK1/2, the same filter was blotted again with the anti-ERK1/2 antibody.

Reverse Transcription−Polymerase Chain Reaction (RT−PCR) and Southern Blot Analysis. KU812 cells were incubated with or without 25 μM flavones for 24 h. Total RNA was extracted from KU812 cells using Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. First-strand cDNA was synthesized from total RNA (10 μg) with a $(dT)_{20}$ primer and 20 U of Moloney mouse leukemia virus (MMLV)-reverse transcriptase (Amersham Pharmacia Biotech). Denaturation at 95 °C for 1 min was followed by primer annealing at 60 °C for 1 min and extension at 72 °C for 1 min. A final extension phase of 7 min was added. Specific primer sequences for each gene were as follows: human FcεRI α chain, sense 5′-GACAGTG-GAGAATACAAATGTCA-3′, and antisense 5′-CTTAGGATGTGGGT-TCAGAAGT-3′; human FcεRI γ chain, sense 5′-GATGATTCCAG-CAGTGGTCTTGCT-3′, and antisense 5′-TAGGGCCAGCTGGTGTT-AATGGCA-3′; human G3PDH, sense 5′-GCTCAGACACCATGGG-GAAGGT-3′, and antisense 5′-GTGGTGCAGGAGGCATTGCTGA-3′. The PCR cycle numbers used for the amplification of the respective cDNAs are 15 for FcεRI α chain and G3PDH and 20 for FcεRI γ chain. Specific PCR fragments were separated on an agarose gel and transferred onto a Hybond-N^+ membrane (Amersham Pharmacia Biotech) and hybridized with probes specific for either the FcεRI α or γ chain, or G3PDH, respectively. The hybridized probes were detected using the Gene Images detection kit (Amersham Pharmacia Biotech).



Chrysin Apigenin

Figure 1. Chemical structures of chrysin and apigenin.

RESULTS

Effect of Flavones on the Cell Surface Expression of the High-Affinity IgE Receptor FcεRI. In this study, we addressed whether chrysin and apigenin, which are in the flavone family (shown in **Figure 1**), had an inhibitory effect on the cell surface expression of FcεRI in human basophlic cells. The surface expression level of FcεRI in KU812 cells cultured with chrysin or apigenin for 24 h was determined by flow cytometer (**Figure 2**). Chrysin and apigenin both were shown to suppress the cell surface expression of FcεRI at more than 10 μM. The suppressive effect on the FcεRI expression was revealed at 3 h after addition of either flavone and was sustained for a period of at least 24 h (**Figure 3**). The mean fluorescence intensity of apigenin-treated cells showed a lower amount of FcεRI expression (11.0) as compared to chrysin treated-cells (16.2). These results suggest that the suppressive effect of apigenin on FcεRI expression was stronger than chrysin. Because the suppressive effect of these flavones was more pronounced after a 24-h culture than a 3-h culture, we examined the effects of flavonoids treatment on FcεRI expression at 24 h for all further experiments.

Effect of Flavones on Total Cellular FcεRI α and γ Chains Protein Levels. To elucidate the molecular mechanism by which these flavones are able to suppress the cell surface expression of FcεRI, we next examined the level of total cellular protein for the FcεRI α and γ chains. After treatment with either of the flavones (25 μM) for 24 h, the level for the FcεRI α and γ chains was assessed by immunoblotting with the anti-FcεRI α or anti-FcεRI γ chain antibody. The amount of total cellular protein for the FcεRI α and γ chains was reduced by treatment with either of the flavones (**Figure 4**). The reduction of the α chain protein level by apigenin treatment was stronger than chrysin. This result agreed with the results obtained by flow cytometry analysis. The reduced level of FcεRI α chain protein may be associated with the difference in the suppressive effect between the two flavones on the cell surface expression of FcεRI.

Effect of the Flavones on FcεRI α and γ Chains mRNA Expression. We next investigated if suppression of FcεRI expression by the flavones is due to a decrease of transcription activity. KU812 cells were cultured for 24 h with either of the flavones, and total RNA was isolated. The mRNA levels of the α and γ chains were measured by RT-PCR and followed by Southern blotting. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was monitored as an internal standard. As shown in **Figure 5**, mRNA expression of both the α and γ chains was reduced by the treatment with either of the flavones. These results suggested that the suppressive effect of chrysin and apigenin is due to the reduction of mRNA for the FcεRI α and γ chains.

Suppressive Effect of Flavones on ERK1/2 Phosphorylation. We previously found that PD98059, which specifically

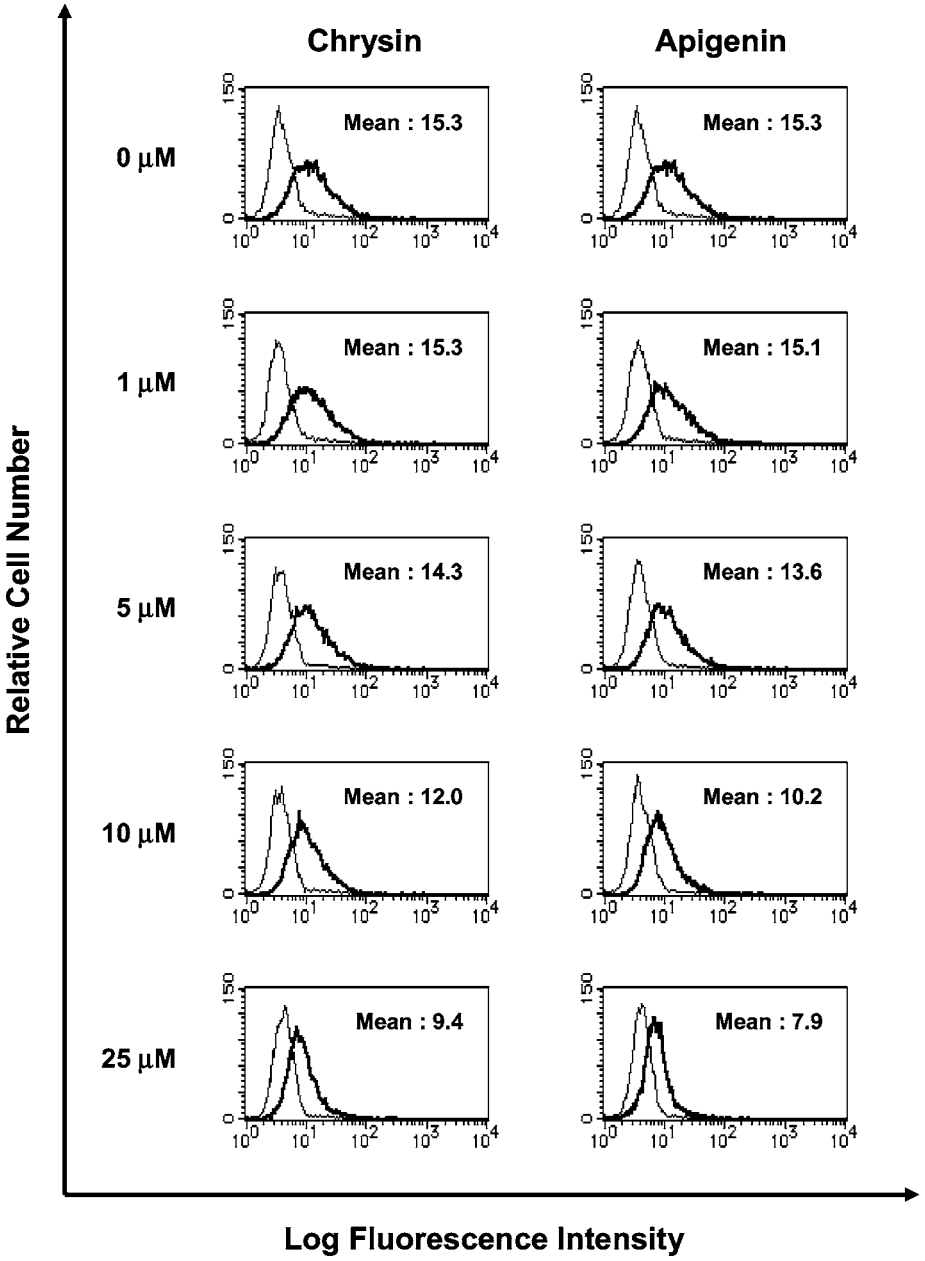


Figure 2. Dose-dependent effect of flavones on the cell surface expression of FcεRI. KU812 cells were cultured with either chrysin or apigenin for 24 h under serum-free conditions. Cells were examined by immunolabeling using the anti-FcεRI α chain antibody followed by staining with the FITC-conjugated goat antimouse IgG antibody. Mouse IgG2b antiboty was used as the isotype-matched negative control. Data acquisition was performed on a FACSCalibur flow cytometer.

inhibits phosphorylation of ERK1/2 by MAPK/ERK kinase1 (MEK1), reduced FcεRI expression (*16*). Thus, we next addressed the expression and phosphorylation of ERK1/2 in KU812 cells treated with 25 μM of either chrysin or apigenin for 1, 6, and 24 h. While there was no change in total ERK1/2 protein level after the treatment with either of the flavones, a reduction of phosphorylated ERK1/2 was observed 6 h after treatment (**Figure 6**). The reduced level of ERK1/2 phosphorylation resulting from treatment with either chrysin or apigenin correlated well with the reduction of phosphorylation induced by PD98059. This suggests that the downregulation of FcεRI expression by chrysin and apigenin is associated with a reduction of phospholyrated ERK1/2.

Inhibitory Effect of Flavones and PD98059 on the Cell Surface Expression of FcεRI. In a previous report, we found that the suppressive effect of PD98059 on the cell surface expression of FcεRI is related to the downregulation of expression of the FcεRI α and γ chain genes (*16*). These results suggest that the action of inhibition of FcεRI expression resulting from treatment with the flavones tested (as shown in **Figure 5**) is similar to PD98059. However, when comparing the FcεRI-suppressive effect of these flavones with PD98059 (**Figure 7**), the suppressive effect of these flavones was stronger than PD98059. These results suggested that the FcεRI-suppressive effect of these flavones may be mediated in part by flavone-induced other activities.

DISCUSSION

Flavonoids are naturally occurring plant polyphenols found in abundance in diets rich in fruit, vegetables, and plant-derivatives such as tea. We previously demonstrated that one

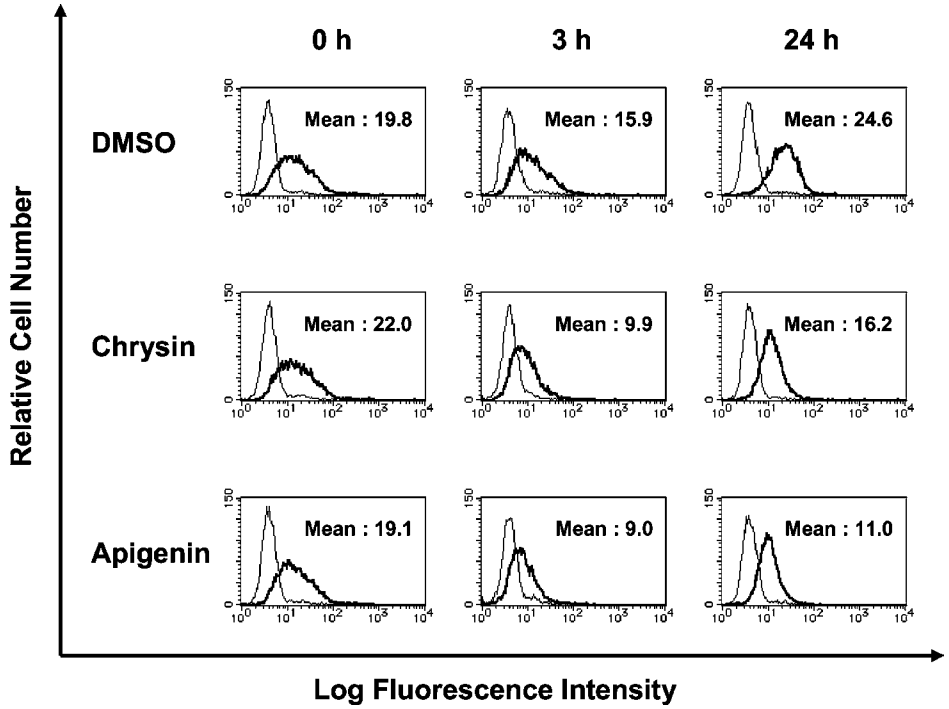


Figure 3. Time course analysis of either chrysin or apigenin on the cell surface expression of FcεRI. KU812 cells were cultured with 25 μM of one or the other flavone for 0, 3, and 24 h under serum-free conditions. Flow cytometric analysis of FcεRI expression was done as described for **Figure 2**.

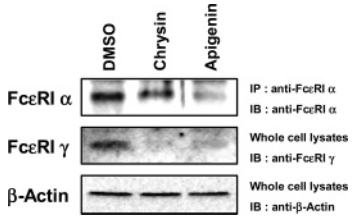


Figure 4. Immunoblot (IB) analysis of the expression of the cellular FcεRI α and γ chain proteins in KU812 cells treated with either chrysin or apigenin. Cells were treated with either flavones (25 μM) under serum-free conditions for 24 h. Whole cell lysates from treated cells were subjected to immunoprecipitaion (IP) using the anti-FcεRI α chain antibody. Precipitated FcεRI α chain and cell lysates were fractionated by 10% or 15% SDS–PAGE and then immunoblotted using the anti-FcεRI α or anti-FcεRI γ chain antibody.

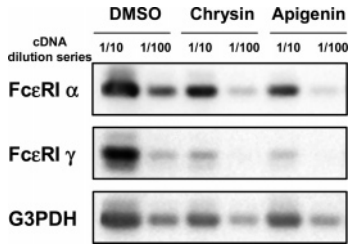


Figure 5. Analysis of the mRNA level for FcεRI α and γ chains. KU812 cells were cultured in the presence of either chrysin or apigenin (25 μM) for 24 h under serum-free conditions. After total mRNA isolation from KU812 cells was performed, FcεRI α, γ, and G3PDH mRNA were analyzed by RT–PCR. Southern blotting using specific probes for FcεRI α, γ, and G3PDH was performed to assess the PCR products.

of the tea polyphenols, (−)-epigallocatechin-3-*O*-gallate (EGCG), can suppress FcεRI expression and has potent antiallergic activity (*15*, *17*). EGCG decreased both protein and mRNA expression of the FcεRI α and γ chains. Here, we show that chrysin and apigenin also suppress FcεRI expression by

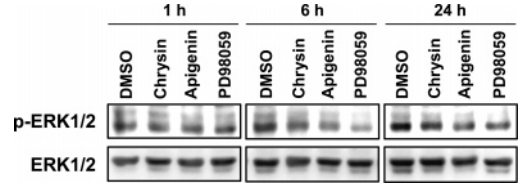


Figure 6. Effects of flavones on ERK1/2 phosphorylation in KU812 cells. KU812 cells were cultured in the presence of 25 μM of either chrysin or apigenin or 10 μM PD98059 for 1, 6, and 24 h under serum-free conditions. The cell lysates were prepared and subjected to 10% SDS–PAGE. Phosphorylated ERK1/2 was detected by immunoblotting using the anti-phospho-ERK1/2 or anti-ERK1/2 antibody.

demonstrating the decrease of both protein and mRNA levels of the FcεRI α and γ chains similar to EGCG. The regulatory mechanism of FcεRI is not fully understood. We demonstrated here that chrysin and apigenin reduced the transcripts of both the FcεRI α and γ chains, as shown in **Figure 4**. FcεRI α chain expression is regulated by at least two transcription factors, GATA-1 and Elf-1, in rodents and other mammals including humans (*18*). To better understand the downregulation mechanism of FcεRI expression by these flavones, it is necessary to examine the involvement of these transcription factors.

We previously demonstrated that the phosphorylation of ERK1/2 is associated with the expression of FcεRI in KU812 cells (*16*). In fact, the two flavones used in this study reduced ERK1/2 phosphorylation. These results suggest that the FcεRI-suppressive effect of the flavones is caused by the reduction of phosphorylated ERK1/2. On the other hand, our results also suggest that the suppressive effect of chrysin and apigenin on FcεRI expression might be mediated in part by other activities of the flavones because both flavones suppressed the cell surface expression of FcεRI stronger than PD98059 as shown in **Figure 7**. We have previously shown that FcεRI expression is negatively regulated by the peroxisome proliferators-activated receptor (PPAR γ) ligand, prostaglandin D_2 metabolite 15 deoxy-$\Delta^{12,14}$

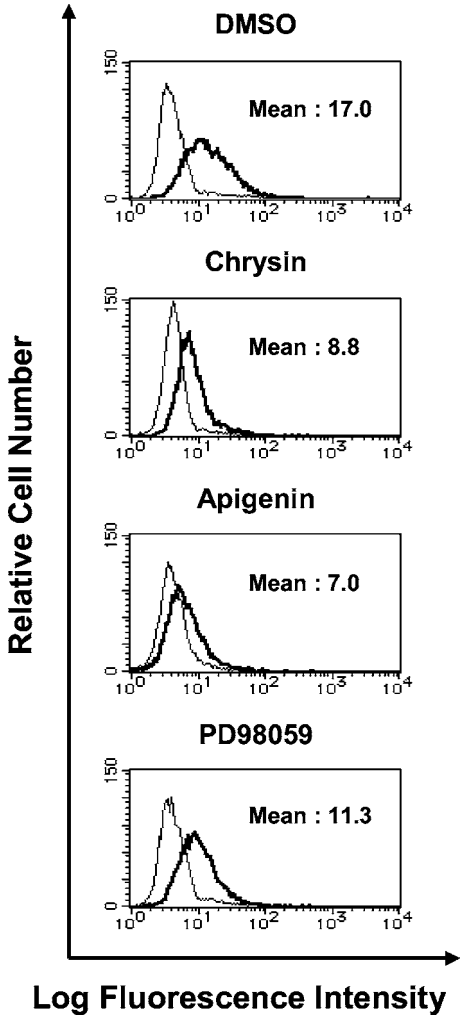


Figure 7. Inhibitory effect of PD98059 and either chrysin or apigenin on the cell surface expression of FcεRI and ERK1/2 phosphorylation. KU812 cells were cultured in the presence of PD98059 (10 μM) or in either of the flavones (25 μM) for 24 h under serum-free conditions. Flow cytometric analysis of FcεRI expression was done as described in **Figure 2**.

prostaglandin J_2 (15d-PGJ_2) (*19*). PPAR γ is activated by the 15d-PGJ_2 and by synthetic antidiabetic thiazolidinedione drugs (e.g., BRL49653 and ciglitizone). Activated PPAR γ negatively regulates the expression of proinflammatory genes and suppresses tumor cell growth (*20*−*22*). Some flavonoids have been considered to be able to bind to PPAR γ and activate it (*23*). These findings raise the possibility that flavonoids could act as a PPAR γ agonist and may potentially have antiallergic activity. Not only the reduction of ERK1/2 phosphorylation but also other activity, such as the regulation of PPAR γ, may be involved in the downregulation of FcεRI when treated with chrysin or apigenin. These results suggest that chrysin and apigenin may have action similar to the PPAR γ ligands. For the future, we will intend to examine the PPAR γ ligand activity of these flavones.

We recently found that the 67 kDa laminin receptor is a molecular target for the green tea polyphenol EGCG (*24*). It will also be important to identify the molecular targets of these flavones to better elucidate the mechanism of the downregulation of FcεRI expression induced by these flavones.

In summary, we show that the strong suppression of FcεRI expression is induced by the addition of either chrysin or apigenin. These results suggest that flavonoids have therapeutic potential that may be useful to inhibit various allergy diseases.

ACKNOWLEDGMENT

The authors thank Perry Seto for proofreading the manuscript.

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Received for review December 9, 2004. Accepted January 6, 2005. This work was supported in part by grants from the Kanpou Science Foundation and from the Integrated Research on Safety and Physiological Function of Food, MAFF, Japan, to H.T.

JF047929D