The effects of four ellagitannin metabolites (M1–M4) and ellagic acid on immunoglobulin E-mediated allergic responses in rat basophilic leukemia-2H3 cells were investigated. M1–M4 inhibited the antigen-induced degranulation and secretion of interleukin-4 and tumor necrosis factor-α, but ellagic acid only slightly did so under the same experimental conditions. M1 inhibited the activation of the mitogen-activated protein kinases in antigen-stimulated cells.

**Key words:** ellagitannin; metabolites; immunoglobulin E; allergy; rat basophilic leukemia-2H3 cells

Ellagitannins (ETs) are natural polyphenols abundant in certain fruits and nuts, including pomegranates, raspberries, and walnuts. They are not absorbed in humans, but are hydrolyzed to yield ellagic acid (EA), which is further metabolized by colon microflora to produce ET metabolites (mainly urolithin A). To characterize the chemical structures of ET metabolites, Ito et al. investigated urinary and intestinal microbial metabolites appearing in rats after ingestion of a purified ellagitannin, geraniin. Four (M1–M4) were major metabolites found in serum and urinary excretion in the rat. To our knowledge, no effects of ET metabolites on IgE-mediated allergic responses have been reported. In the present study, we used the rat basophilic leukemia (RBL–2H3) cell line, which has been used extensively as an in vitro model for the study of FcεRI signaling, to investigate the effects of M1–M4 on IgE-mediated allergic responses in comparison with EA.

M1–M4 were prepared as described previously. EA and quercetin were purchased from Sigma-Aldrich (St. Louis, MO). The polyphenol samples were dissolved in dimethyl sulfoxide (DMSO) for use in the experiments. RBL-2H3 cells were maintained in Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich) with 10% fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in a humidified atmosphere containing 5% CO2.

We measured antigen (Ag)-induced β-hexosaminidase release from the RBL-2H3 cells to study the ability of the metabolite compounds to inhibit degranulation activity. The cells were seeded in a 24-well plate (2.5 × 105/well) and cultured overnight. They were sensitized with DNP-specific IgE at 50 ng/mL for 2 h. After the cells were washed with modified Tyrode (MT) buffer containing 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 20 mM HEPES, and 0.1% BSA at pH 7.4, polyphenol samples diluted in MT buffer were added. After 10 min of incubation, DNP-HSA (final concentration 50 ng/mL) was added, and the culture was incubated for 30 min. The supernatant was collected, and the cells were lysed with MT buffer containing 0.1% Triton X-100. Aliquots of each supernatant and cell lysate were incubated with 1 mM p-nitrophenyl-N-acetyl-β-D-glucosamide solubilized in 0.05 M citrate buffer (pH 4.5) for 30 min at 37°C. The enzyme reaction was terminated by the addition of 0.05 M sodium carbonate buffer (pH 10), and the absorbance (at 415 nm) was measured. Figure 1 shows the inhibitory effects of EA, quercetin, and the four ET metabolites on Ag-induced β-hexosaminidase release from the cells. Each of the ET metabolites and quercetin significantly and dose-dependently reduced β-hexosaminidase release. Ag-induced β-hexosaminidase release was inhibited in rank order M3 > M1 > M2, quercetin, M4. The IC50 values for M1, M2, M3, M4, and quercetin were 2.1, 4.9, 0.7, 5.7, and 4.9 μM, respectively. In contrast, EA only slightly inhibited Ag-induced β-hexosaminidase release, by 12 ± 3% at a concentration of 32 μM.

The ET metabolites, but not EA itself, significantly inhibited Ag-induced degranulation in the cells. M1 and M3 showed the strongest inhibitory activity, and quercetin, M2 and M4 had slightly smaller effects. These results are in keeping with previous reports. Itoh et al. reported that EA inhibited Ag-induced β-hexosaminidase release from RBL–2H3 cells in a dose-dependent manner at concentrations of 5–50 μM, but, in agreement with our own findings, its inhibitory activity was lower than that of quercetin. In addition, Choi and Yan found that oral administration of EA inhibited anti-DNP IgE-mediated passive cutaneous anaphylaxis in rats. These results suggest that the in vivo activity of
EA on the IgE-mediated degranulation of basophils and mast cells is due to metabolites generated by colon microflora.

We also determined the effects of the four metabolites and EA on the viability of RBL-2H3 cells incubated with the four metabolites and with EA at 32 \( \mu M \) for 24 h. Viability was assessed using an MTT cell proliferation assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).

When Ag crosslinks adjacent IgE molecules, aggregation of FcεRI triggers a complex intracellular signaling process that results in the secretion of allergy-related cytokines.\(^5\) Hence we examined to determine whether ET metabolites and EA can modulate Ag-induced secretion of IL-4 and TNF-\( \alpha \) in RBL-2H3 cells (Fig. 2). Cells were seeded into a 24-well culture plate and cultured overnight. They were sensitized with DNP-specific IgE, incubated with 0.1% DMSO (control) or a polyphenol sample in MT buffer, and then challenged with DNP-HAS, as described above. Four h after challenge with DNP-HSA, the supernatant was collected, and cytokine levels were determined by ELISA assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer’s instructions. No significant differences were found (data not shown).
In summary, our data provide evidence that bioavailable ET metabolites inhibit IgE-mediated allergic responses in an in vitro model system. Four major metabolites (M1–M4) intensely suppressed Ag-induced degranulation and the secretion of allergy-related cytokines (IL-4 and TNF-α) in RBL-2H3 cells. In contrast, EA had little inhibitory effect on degranulation and no effect on cytokine secretion under these assay conditions. It is noteworthy that M1 inhibited the activation of JNK, ERK, and p38-MAPK, suggesting that inhibition of IgE-mediated allergic responses can be mediated by inhibition of MAPK pathways. Further research is warranted to verify these results in vivo, but our results highlight that ET metabolites, generated following dietary consumption of ET-rich foods such as pomegranates and walnuts, may be therapeutically valuable in preventing allergic symptoms by inhibiting mast cell activation.

Acknowledgments

We thank the staff of the Tissue Culture Research Center of Kawasaki Medical School. This investigation was supported by the Research Fund of Kawasaki University of Medical Welfare (beneficiary, T. N.).

References