Original article

UDP/P2Y6 receptor signaling regulates IgE-dependent degranulation in human basophils

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P2YR, P2Y purinergic receptors; GPCR, G-protein coupled receptor; ENTPDase, ecto-nucleoside triphosphate diphosphohydrolase; AC, adenylate cyclase; PLC, phospholipase C; PKC, protein kinase C; MFI, mean fluorescence intensity; 7-AAD, 7-amino-actinomycin D; ENPP, ecto-nucleotide pyrophosphatase/phosphodiesterase

A B S T R A C T

Background: P2Y purinergic receptors (P2YR) are G-protein coupled receptors that are stimulated by extracellular nucleotides. They mediate cellular effects by regulating cAMP production, protein kinase C activation, inositol trisphosphate generation, and Ca2+ release from intracellular stores. The P2Y6 receptor of this family is selectively stimulated by UDP, and selectively inhibited by MRS2578. In the present study, we examined the effect of UDP/P2Y6 receptor signaling on IgE-dependent degranulation in human basophils.

Methods: Basophils were purified from human peripheral blood. The mRNA expression of genes encoding P2YR and ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) was measured by RT-PCR. Intracellular Ca2+ influx via UDP/P2Y6 receptor signaling in basophils was detected using a calcium probe. The effect of UDP/P2Y6 receptor signaling on IgE-dependent degranulation in basophils was confirmed by measuring CD63 expression by flow cytometry. Autocrine secretion of nucleotides was detected by HPLC analysis.

Results: We showed that purified basophils express P2Y6 mRNA and that UDP increased intracellular Ca2+, which was reduced by MRS2578 treatment. UDP promoted IgE-dependent degranulation. Furthermore, MRS2578 inhibited IgE-dependent degranulation in basophils. HPLC analysis indicated that basophils spontaneously secrete UTP. In addition, basophils expressed the extracellular nucleotide hydrolases ENTPDase2, ENTPDase3, and ENTPDase8.

Conclusions: This study showed that UDP/P2Y6 receptor signaling is involved in the regulation of IgE-dependent degranulation in basophils, which might stimulate the P2Y6 receptor via the autocrine secretion of UTP. Thus, this receptor represents a potential target to regulate IgE-dependent degranulation in basophils during allergic diseases.

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Introduction

Nucleotides are released from damaged cells, secreted by activated cells, or spontaneously secreted.1,2 Extracellular nucleotides regulate a variety of biological functions through P2 purinergic receptors expressed on cell surfaces.3,4 P2 purinergic receptors are divided into two subfamilies: P2X receptors (P2X1-7), which function as ion channels, and P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14), which function as G protein-coupled-receptors (GPCRs). The ligands of P2Y receptors vary according to subtype. ATP is the ligand for P2Y2 and P2Y11, ADP for P2Y1, P2Y12, and P2Y13, UTP for P2Y2 and P2Y4, UDP for P2Y6, and UDP-glucose for P2Y14. The effects of P2Y receptor activation vary according to the coupled G protein. The Gαq-coupled receptor subtype (P2Y11) activates adenylate cyclase (AC). Conversely, Gα1 coupled receptor subtypes (P2Y12, P2Y13, and P2Y14) inhibit AC. The Gα1-coupled receptor subtypes (P2Y1, P2Y2, P2Y4, and P2Y6) activate phospholipase C (PLC) to increase intracellular Ca2+ and activate protein kinase C (PKC)5–7.

The P2Y6 receptor is distributed in immune cells and non-immune cells and is selectively stimulated by UDP and selectively inhibited by MRS2578. Mast cells are activated by P2Y6 UDP/P2Y6 receptor signaling regulates IgE-dependent degranulation in human basophils...
receptor stimulation to release MIP-1β. The P2Y6 receptor inhibits the activation of CD4+ T cells and protects against allergic pulmonary inflammation. During allergic airway inflammation and allergic bronchospasm, increased P2Y6 receptor expression in epithelial cells is involved in the control of inflammation and remodeling. In addition, microglial phagocytosis is accelerated by UDP stimulation via the P2Y6 receptor. By activating PKC, UDP stimulation of the P2Y6 receptor also protects against apoptosis induced by TNF secretion by astrocytoma cells. However, to date, there have been no reports regarding the effects of P2Y6 on basophil function during allergic inflammation.

Activated basophils release histamine and leukotriene and produce cytokines such as IL-4 and IL-13; this has an important role in allergic reactions and biological defense against parasitic infections. The signal transduction associated with IgE-dependent and IgE-independent signaling in basophils is similar to intracellular signaling of the Gαq protein through the P2Y2 receptor. IgE-dependent activation induced by an allergen results in PKC activation and intracellular Ca2+ increase. The MCP-1 receptor, C5a receptor, and fMLP receptor induce IgE-independent activation of basophils via the GPCR, which induces an increase in intracellular Ca2+ and activation of PKC.

It was reported that Gzα12/13, which is induced through the regulation of RhöGEF, as well as increased cAMP and PKA activation, are associated with the P2Y6 receptor. If the G protein associated with the P2Y6 receptor is Gαq, stimulation of this receptor results in degranulation of basophils via PKC activation and intracellular Ca2+ increase, thus enhancing IgE-dependent and IgE-independent basophil degranulation. If the associated G protein is Gα12/13, stimulation increases intracellular Ca2+ and enhances IgE-dependent and IgE-independent basophil degranulation. Therefore, it was expected that stimulation of UDP/P2Y6 receptor signaling would induce the degranulation of basophils. However, to date, there have been no reports regarding the effects of the P2Y6 receptor on basophil function.

In this study, we demonstrated that stimulation of the UDP/P2Y6 receptor affected IgE-dependent degranulation of basophils. These cells produced UDP spontaneously, possibly via constant P2Y6 stimulation. Furthermore, degranulation of basophils was inhibited by MRS2578. Taken together, our findings showed that the P2Y6 receptor might be a potential target for the regulation of IgE-dependent degranulation in basophils.

**Methods**

**Basophil isolation**

Peripheral blood samples were taken from 10 healthy volunteers after obtaining informed consent. Subjects ranged in age from 20 to 38 years. Peripheral blood (10–20 ml) was collected and basophils were isolated using Hetasep™ and Easysep™ Human Basophil Enrichment kits (StemCell Technologies, Vancouver, Canada), according to the manufacturer’s instructions. The purity of enriched basophils was determined by flow cytometry using a Cytomics FC500 (Beckman Coulter, Fullerton, CA, USA) with APC-conjugated anti-CD123 (Miltenyi Biotec, Bergisch Gladbach, Germany) and FITC-conjugated anti-FcεRI (BioLegend, San Diego, CA, USA). The resulting purity was determined to be >94% (Supplementary Fig. 1). This study was approved by The Committee for Medical Ethics of Hirosaki University Graduate School of Medicine, Hirosaki, Japan.

**RNA extraction and reverse transcription PCR analysis**

Total RNA was extracted from 1 × 10⁵ cells using a total RNA isolation Nucleospin® RNA XS kit (MACHEREY-NAGEL, Düren, Germany). Total RNA was reverse transcribed using the PrimeScript™ II 1st strand cDNA Synthesis Kit (TaKaRa, Bio, Shiga, Japan). P2Y receptor amplifications were performed with 1 μl of cDNA in GoTaq® Green Master Mix solution (Promega, Madison, WI, USA) using a thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The cycle conditions were as follows: 3 min at 94 °C and 35 cycles of 1 min at 94 °C, 1 min at the appropriate annealing temperature (P2Y1, 2, 12, 13 = 55 °C, P2Y4, 6, 11 = 62.5 °C, P2Y14 = 60 °C, GAPDH = 58 °C), and 1 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C and a final 7 min step at 72 °C. Ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) amplifications were performed with 1 μl of cDNA in GoTaq® Green Master Mix solution using a Thermal cycler (Bio-Rad Laboratories). The cycle conditions were as follows: 3 min at 94 °C and 20 cycles of 1 min at 94 °C, 1 min at 75 °C (and then decreasing by 1 °C/cycle), and 1 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C and a final 7 min step at 72 °C. The primer sequences and expected sizes of the PCR products are shown in Table 1. The primer sequence for P2Y receptor and GAPDH were described by Kudo et al., and the primer sequence of ENTPDase was described by Bahrami et al. PCR products were analyzed after electrophoretic separation using 1.5% agarose gels containing ethidium bromide and were visualized using a ChemiDoc XRS and Quantity One software (Bio-Rad Laboratories).

**Measurement of intracellular Ca2+**

Cellular concentrations of Ca2+ were measured using the Calcium Kit II – iCellux (Dojindo, Kumamoto, Japan). Isolated basophils were incubated at 1 × 10⁶ cells/ml in HEPES-HBSS supplemented with a Ca2+ probe and 1.25 mM probenecid for 60 min at 37 °C and 5% CO2. After washing twice with HEPES-HBSS, cells were suspended at 1 × 10⁶ cells/ml in HEPES-HBSS or HEPES-HBSS with 10 μM MRS2578 (Sigma Aldrich, St. Louis, MO, USA). Subsequently, 100 μM of UDP and 0.1 μg/ml anti-IgE were added to calcium probe-labeled cells and fluorescence measurements of Ca2+ were performed using a TriStar LB941 (Berthold Technologies, Germany).

**Basophil degranulation**

Isolated basophils were suspended at 1 × 10⁵ cells/ml in HBSS with HEPES (StemCell Technologies). Basophils were stimulated with HEPES-HBSS or HEPES-HBSS with 10 μM MRS2578 (Sigma Aldrich, St. Louis, MO, USA). 3 min at 94 °C, 1 min at the appropriate annealing temperature (P2Y1, 2, 12, 13 = 55 °C, P2Y4, 6, 11 = 62.5 °C, P2Y14 = 60 °C, GAPDH = 58 °C), and 1 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C and a final 7 min step at 72 °C. Ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) amplifications were performed with 1 μl of cDNA in GoTaq® Green Master Mix solution using a Thermal cycler (Bio-Rad Laboratories). The cycle conditions were as follows: 3 min at 94 °C and 20 cycles of 1 min at 94 °C, 1 min at 75 °C (and then decreasing by 1 °C/cycle), and 1 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C and a final 7 min step at 72 °C. The primer sequences and expected sizes of the PCR products are shown in Table 1. The primer sequence for P2Y receptor and GAPDH were described by Kudo et al., and the primer sequence of ENTPDase was described by Bahrami et al. PCR products were analyzed after electrophoretic separation using 1.5% agarose gels containing ethidium bromide and were visualized using a ChemiDoc XRS and Quantity One software (Bio-Rad Laboratories).

**Table 1**

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<th>Gene</th>
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<th>Reverse primer</th>
<th>Amplicon (bp)</th>
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with 0.1 μg/ml of goat monoclonal anti-IgE (Beckman Coulter) and 1 μM to 10 nM of UDP at 37 °C for 15 min. Degranulation was stopped by chilling the samples on ice, after which FITC-conjugated anti-CD63 (BioLegend) antibodies were added with subsequent incubation for 30 min on ice. Mean fluorescence intensity (MFI) of CD63 in basophils after stimulation was measured by flow cytometry. All basophils were acquired using a Cytomics FC500 and analysis was performed using FlowJo software (Tree Star, Ashland, OR, USA).

MRS2578 treatment of basophils

Isolated basophils were cultured at 1 × 10^5 cells/ml in RPMI1640 containing 5% serum from the blood sample donor, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 ng/ml recombinant human IL-3 (Wako Pure Chemical, Osaka, Japan). Basophils were treated in 24-well plates with 10 μM MRS2578 for 0.5, 18, and 42 h. Controls were treated with 0.1% DMSO. Cultured basophils were suspended at 1 × 10^5 cells/ml in HEPES-HBSS with 10 μM MRS2578 or 0.1% DMSO. Cultured basophils were stimulated using 0.1 μg/ml anti-IgE, and the MFI of CD63 in basophils was measured by flow cytometry. All basophils were acquired using a Cytomics FC500 and analysis was performed using FlowJo software.

Viability assay

Apoptosis and necrosis were detected using APC-conjugated annexin V and 7-amino-actinomycin D (7-AAD) assays. Briefly, basophils that were cultured with 0.1% DMSO or 10 μM MRS2578 for 18 or 42 h were suspended in Annexin V Binding Buffer and stained with APC-conjugated Annexin V and 7-AAD (BioLegend) for 30 min on ice. All basophils were acquired using a Cytomics FC500 and analysis was performed using FlowJo software.

Detection of spontaneous nucleotide secretion from basophils by HPLC analysis

Isolated basophils were cultured at 1 × 10^5 cells/ml in RPMI1640 medium containing 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO2 for 18 h. The supernatant was separated from the basophil culture medium by centrifugation at 14,000 × g for 5 min, and maintained at −20 °C until use. Reversed-phase HPLC was performed using a Hypersil ODS C18 column (150 mm × 4.6 mm, particle size, 5 μm; YMC, Kyoto, Japan). The column was eluted with 100 mM KH2PO4–K2HPO4 (pH 5.5) at a flow rate of 1.0 ml/min and nucleotides were detected at 260 nm using a Hitachi L-7100 (Hitachi High-Technologies, Tokyo, Japan).

Statistical analysis

Data are presented as the means ± SEM. Statistical significance was determined using a paired Student’s t-test. *P < 0.05 was considered statistically significant.

Results

UDP stimulates the P2Y6 receptor in basophils

The expression of P2Y receptors on basophils had not previously been confirmed. RNA was extracted from isolated human basophils, and P2Y receptor mRNA expression was confirmed by RT-PCR. In five independent experiments, bands were detected by electrophoresis for P2Y2, P2Y4, P2Y6, P2Y12, P2Y13, and P2Y14 (Fig. 1A). Therefore, it was hypothesized that the basophils would be responsive to UDP, which is a ligand of the P2Y6 receptor.

UDP stimulation through the P2Y6 receptor increased intracellular Ca2+. We confirmed the change in intracellular Ca2+ in basophils following UDP stimulation (three independent experiments). Intracellular Ca2+ in basophils was immediately increased by UDP stimulation (Supplementary Fig. 2) and was significantly inhibited by MRS2578 (Fig. 1B). The fluorescence intensity was increased to 1305.5 ± 35.6 with UDP stimulation, and reduced to 251.7 ± 81.3 through the addition of MRS2578. The increased fluorescence intensity after UDP stimulation was decreased by 80.7% with MRS2578 treatment. The increased fluorescence intensity with anti-IgE stimulation was similar to that after UDP stimulation (1207.6 ± 344.6). Therefore, UDP has the potential to induce degranulation and enhance IgE-dependent degranulation in basophils.

UDP enhances IgE-dependent degranulation of basophils

Based on previous results, it was suggested that UDP might induce degranulation and enhance IgE-dependent degranulation in human basophils. We confirmed the effect of UDP stimulation on degranulation by measuring CD63 expression (three independent experiments). CD63 is known as a granular-associated molecule. Basophils incubated with UDP for 15 min exhibited expression of CD63. There was no significant difference in the MFI of CD63 after UDP stimulation, regardless of concentration, when compared to that in the unstimulated control (Fig. 2A).

Next, we confirmed the effect of UDP/P2Y6 receptor signaling on IgE-dependent degranulation in basophils. Basophils incubated with UDP and anti-IgE for 15 min exhibited CD63 expression. IgE-
dependent degranulation reached its maximum with 100 pM UDP (Fig. 2B). There was a statistically significant difference in the MFI of CD63 in basophils stimulated with anti-IgE (21.4 ± 1.4) compared to that in cells treated with 100 pM UDP and anti-IgE (31.6 ± 2.7).

Basophils secrete UTP

Some immune cells secrete UDP spontaneously, and activated basophils secrete ATP.\textsuperscript{31,32} We confirmed the presence of nucleotides in the culture supernatant of basophils by HPLC, even when the basophils had not been activated by secreted nucleotides (three independent experiments). In the culture supernatant of basophils, a peak was confirmed at a retention time of 2.33 min (Fig. 3A). The retention time of ATP was 6.30 min, that of ADP was 7.75 min, that of UTP was 2.32 min, that of UDP was 2.53 min, and that of UDP-glucose was 2.87 min (Fig. 3B). Nucleotides were not detected in the RPMI1640 medium (Fig. 3C). These findings confirmed that basophils secrete UTP.

Expression of ENTPDase in basophils

ENTPDase and ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) are known as extracellular nucleotidic hydrolases. CD203c, a marker of basophils, is an ENPP3 that hydrolyzes nucleotides\textsuperscript{33,34} such as ATP. We confirmed the mRNA expression of ENTPDase1, ENTPDase2, ENTPDase3, and ENTPDase8, which are extracellular nucleotide hydrolases, in human basophils by RT-PCR (Fig. 4). In three independent experiments, bands were detected by electrophoresis for ENTPDase2, ENTPDase3, and ENTPDase8; these enzymes hydrolyze nucleotide triphosphate to nucleotide diphosphoric acid. However, the expression of ENTPDase1, which hydrolyzes nucleotide triphosphate and nucleotide diphosphoric acid to nucleotide monophosphate, was not confirmed.\textsuperscript{35} Therefore, UTP secreted by basophils might be hydrolyzed to UDP by ENTPDase2, ENTPDase3, and ENTPDase8.

MRS2578 inhibits IgE-dependent degranulation in basophils

Our findings suggested that basophil P2Y6 receptors might be stimulated by the spontaneous production of UDP. Even when UDP was not added exogenously, basophils continuously produced UDP, which could enhance IgE-dependent degranulation. We confirmed the IgE-dependent degranulation of basophils by inhibiting the P2Y6 receptor and measuring the expression of CD63. Basophils were cultured with MRS2578, a specific antagonist of P2Y6 receptor, for 0.5, 18, and 42 h. Controls consisted of treatment with the MRS2578 vehicle solution, DMSO (three independent experiments). Basophils cultured with MRS2578 showed decreased expression of CD63 following anti-IgE stimulation compared to that

Fig. 2. Effects of UDP on IgE-dependent basophil degranulation. After stimulating basophils, the mean fluorescent intensity of CD63 was measured by flow cytometry. (A) Basophils were stimulated for 15 min with 1 pM–10 nM UDP (n = 3). Data represent the mean ± SEM (n = 3); statistical significance was assessed by a Student’s t-test. A significant difference compared to medium alone was not observed. (B) Basophils were stimulated with 1 pM–10 nM UDP and 0.1 μg/ml anti-IgE (n = 3). Data represent the mean ± SEM (n = 3); statistical significance was assessed by performing a Student’s t-test. *P < 0.05, significantly different from the value of the anti-IgE group.

Fig. 3. Spontaneous secretion of nucleotides. Nucleotides in the culture supernatant of basophils were detected by reversed-phase HPLC. Each peak was identified by comparison with the retention times of a standard mixture. (A) Culture supernatant; (B) nucleotide standard mixture; (C) RPMI1640 medium only (representative of three experiments).

Fig. 4. Expression of ENTPDase mRNA. The mRNA expression of ENTPDase family members and GAPDH in human basophil was evaluated by RT-PCR (representative of three experiments).
in basophils cultured with DMSO (Fig. 5). The MFI of CD63 on basophils following anti-IgE antibody stimulation was 18.6 ± 0.8 at 0.5 h, 16.4 ± 0.3 at 18 h, and 1.7 ± 0.2 at 42 h, with MRS2578 treatment. The MFI of CD63 on basophils following anti-IgE antibody stimulation was 20.0 ± 0.4 at 0.5 h, 22.4 ± 0.3 at 18 h, and 21.5 ± 0.1 at 42 h, with DMSO treatment. Thus, MFI decreased by 7.0% at 0.5 h, 26.8% at 18 h, and by 92.2% at 42 h with MRS2578 treatment. This confirmed that MRS2578 inhibits the IgE-dependent degranulation of basophils, indicating that the IgE-dependent degranulation of basophils is regulated by UDP via the P2Y6 receptor. MRS2578 does not alter the survival of basophils

We then examined the cytotoxicity of MRS2578 using Annexin-V and 7-AAD (three independent experiments). The number of cells negative for Annexin-V and 7-AAD was 96.9 ± 0.7% following the addition of MRS2578 at 18 h and 97.3 ± 0.4% following the addition of DMSO at the same time point. The number of cells negative for Annexin-V and 7-AAD was 96.3 ± 0.5% following the addition of MRS2578 at 42 h and 97.6 ± 0.2% following the addition of DMSO (Fig. 6). No significant difference in cytotoxicity between the MRS2578 and DMSO groups was observed at either time point.

Discussion

The P2Y6 receptor is involved in the biological activity of various cells, and the aggravation of allergic symptoms is associated with allergic reactions.3–10 However, the effect of the P2Y6 receptor on the function of basophils, which have an important role in allergic reactions, has not been reported. In this study, we showed that stimulation of UDP/P2Y6 receptor signaling enhanced the IgE-dependent degranulation of basophils. Furthermore, we showed that MRS2578, a specific antagonist of the P2Y6 receptor, inhibited IgE-dependent degranulation. Therefore, inhibition of P2Y6 receptor stimulation inhibits the degranulation of basophils, and might be effective for allergy treatment.

Expression patterns of the P2Y receptor in basophils have not been reported to date. In this study, we detected the expression of P2Y receptor mRNA in basophils, and determined that the selective agonistic effect of UDP on P2Y6 receptors increased intracellular Ca2+ in basophils, which was inhibited by incubation with MRS2578. These results indicate that expression of the P2Y6 receptor is present in basophils. However, the P2Y6 receptor protein has not been detected to date and this should be assessed in future studies.

Inhibition of IgE-dependent basophil degranulation by MRS2578 indicates that basophils are constantly stimulated by UDP and that stimulation via the P2Y6 receptor is necessary for IgE-dependent basophil degranulation. IgE-dependent degranulation was enhanced after 15 min of UDP stimulation. In contrast, inhibition of IgE-dependent degranulation by MRS2578 required a reaction time of 18 h or longer. The possibility that the basophils had been constantly stimulated by UDP was considered. One inhibitory mechanism of MRS2578 is to block the P2Y6 receptor and its response to UDP. Therefore, the lack of an effect is due to the fact that UDP stimulation is thought to require 18 h or longer.

Expression of ENTPDase mRNA and the spontaneous secretion of UTP suggested the possibility that the P2Y6 receptor on basophils is stimulated by an autocrine mechanism. The P2Y6 receptor constantly stimulates basophils, and therefore, should have an important role in their degranulation. Furthermore, this receptor reacts minimally with UTP.16 UDP was not detected by HPLC analysis. It was considered that UDP reacted with the P2Y6 receptor or ENTPDase on the cell surface such that UDP might be undetectable in the culture supernatant. Therefore, hydrolysis of UTP by basophils, and detection of UDP and ENTPDase proteins on the cell surface is necessary in further studies to confirm autocrine activation of P2Y6.

Intracellular signaling induces many cell functions. PKC and PLCg signal transduction molecules are Ca2+-dependent.17,21 Furthermore, molecules released during the degranulation of basophils and mast cells that fuse to cell membranes are also Ca2+-dependent.39,40 Therefore, intracellular Ca2+ might have an important role in the activation and degranulation of basophils. Indeed, the IgE-dependent and IgE-independent activation of basophils is induced by the activation of PKC, which increases intracellular Ca2+.17,21

Gq protein-coupled GPCRs induce the activation of PKC, increase intracellular Ca2+, and induce the degranulation of basophils. Furthermore, the C5a receptor, MCP-1 receptor, and fMLP receptor (a GPCR) induce the IgE-independent degranulation of
basophils. It has been suggested that when the G protein coupled with the P2Y6 receptor in basophils is Gαq, degranulation is induced by UDP stimulation. However, UDP stimulation did not induce the degranulation of basophils, herein. It was shown that Gαz13 increased the intracellular Ca2+ necessary for degranulation, but did not activate PKC.12 If Gαz13 is coupled with the P2Y6 receptor in basophils, this might explain why degranulation was not induced by UDP stimulation. Furthermore, these results indicate that Gαz13 promotes IgE-dependent degranulation because an increase in intracellular Ca2+ strengthens FcεRI signaling.17 However, further studies will be necessary to confirm the activation of Rho as well as increases in cAMP via UDP stimulation, as proving that Gαz13 is coupled with the P2Y6 receptor was beyond the scope of this study.

IL-4, which is produced by basophils, is associated with IgE production18 and Th2 differentiation.19 Since this study did not examine the effect of UDP/P2Y6 receptor signaling on cytokine production in basophils, future investigation into the effect of this pathway on cytokine production is necessary.

Basophils have an important role in allergic disease15 and in biophysics against parasitic infections.6 Inhibiting basophil function prevents allergic reactions, but consequently immunity to parasitic infection decreases. Ultimately, UDP/P2Y6 receptor signaling has a role in biophysics against viruses, bacteria, and parasites.31,34,44 Inhibition of UDP/P2Y6 receptor signaling might decrease immunity against infections. Therefore, it is important to determine the effect of inhibition of the P2Y6 receptor, a target for the treatment of allergic disease, on infection. Because this study investigated cellular responses in vitro, investigating the effect of UDP/P2Y6 receptor signaling using in vivo models of allergic reaction and immunity against infections is required in future studies.

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Appendix A Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalit.2017.02.014.

Conflict of interest

The authors have no conflict of interest to declare.

Authors’ contributions

MN, KL, and HT designed the study and wrote the manuscript. MN, TY, NS, SA, KK, and TN contributed to data collection. MN performed the statistical analysis and interpretation of the results. All authors read and approved the final manuscript.

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