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FcεRI, but Not FcγR, Signals Induce Prostaglandin D2 and E2 Production from Basophils

Tsukasa Ugajin,* Takahiro Satoh,*
Toru Kanamori,* Kosuke Aritake,†
Yoshihiro Urade,† and Hiroo Yokozeki*

From the Department of Dermatology, Graduate School, Tokyo Medical and Dental University, Tokyo; and the Department of Molecular Behavioral Biology,† Osaka Bioscience Institute, Osaka, Japan*

Prostaglandin (PG) D2 and PGE2 are arachidonic acid metabolites that are generated though an isomerization reaction catalyzed by PG synthases. PGs have been implicated in immunologic reactions in addition to a wide range of physiological functions. It has long been thought that basophils, in contrast to mast cells, do not synthesize PGs, although they do release leukotrienes and platelet-activating factor. Here, we show that basophils function as a source of PGD2 and PGE2. *In vitro*-cultured basophils from mouse bone marrow produced both PGD2 and PGE2 in response to IgE + antigen (Ag), but not to IgG + Ag. Release of PGs was almost completely abrogated in cultured basophils from FcRγ-chain$^{-/-}$ mice, indicating the involvement of FcεRI. Basophils freshly isolated from bone marrow cells (primary basophils) were also capable of secreting PGD2 and PGE2. Although the amount of PGD2 released from primary basophils was lower than that from mast cells, the capability of primary basophils to generate PGE2 was more potent than that of mast cells. Transcripts and proteins for both hematopoietic-type PGD synthase and PGE synthase were detected in basophils. In addition, human basophils, like mouse basophils, also produced PGD2 through IgE-mediated stimulation. Thus, basophils could be an important source of PGD2/PGE2 and may contribute to allergic inflammation and immune responses. ***(Am J Pathol 2011, 179:775–782; DOI: 10.1016/j.ajpath.2011.04.023)***

Prostaglandins, such as PGD2 and PGE2, are cyclooxygenase (COX) metabolites of arachidonic acids. They have a wide range of biological activities, including relaxation and contraction of smooth muscles, and modulation of neuronal activity.[1] PGD2 is principally produced by activated mast cells and, to a lesser extent, by T helper cell 2 (Th2) cells and dendritic cells[2–4] and exerts its effect through D prostanoid[5] and chemoattractant receptor-homologous molecule receptors expressed onTh2 lymphocytes (CRTH2).[6] A number of recent studies have shown that PGD2 is involved in inflammatory reactions. Mast cell-derived PGD2 suppresses IL-12 production by dendritic cells that induce Th2 responses *in vivo*.[7] PGD2 both activates and induces chemotaxis of Th2 cells, eosinophils, and basophils.[6] A large amount of PGD2 is detected in broncho-alveolar lavage fluid during allergen-induced airway inflammation.[8] Transgenic mice overexpressing human PGD synthase showed exacerbation of ovalbumin (OVA)-induced lung inflammation associated with pronounced eosinophilia and increased Th2 cytokine production.[9] In our previous studies, mice deficient in the *CRTH2* gene were characterized by alleviated IgE-mediated cutaneous responses, contact hypersensitivity reactions,[10] and cedar pollen dermatitis.[11] However, PGE2 is produced by a variety of cells, including fibroblasts and macrophages, and exerts its effects via prostaglandin E2 (EP)1, EP2, EP3, and EP4 receptors.[12] PGE2 acts on T cells to enhance production of Th2-type cytokines and to inhibit production of Th1 cytokines *in vitro*,[13] whereas another study showed that PGE2 facilitates Th1 differentiation via EP2 and EP4 signaling.[14] Furthermore, PGE2 enhances IL-23 production by dendritic cells via EP4[15] and facilitates Th17 expansion in the presence of IL-23 through EP2 and EP4 signaling.[14] Thus, PGD2 and PGE2 could be important mediators in immune and allergic responses.

Basophils represent <1% of peripheral blood leukocytes. Like mast cells, they express the high-affinity IgE receptor, FcεRI, on their cell surface and release chemical mediators after FcεRI cross-linking. Under physiological conditions, basophils principally circulate in periph-

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Address reprint requests to Takahiro Satoh, M.D., Ph.D., Department of Dermatology, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, 113-8519, Japan. E-mail: tasa-1688.derm@tmd.ac.jp.

eral blood, whereas mast cells reside in peripheral tissues. During allergic inflammation, such as bronchial asthma and atopic dermatitis, basophils infiltrate into peripheral tissues.[16,17] Several lines of evidence have shown that basophils play a major role in allergic reactions and/or immune responses.[18–21] Basophils initiate IgE-mediated, chronic, cutaneous allergic inflammation (IgE-CAI).[18] They also elicit IgG- but not IgE-mediated anaphylaxis.[19] Basophils promote antigen-specific Th2 development[20] and augment humoral memory responses.[21] Basophils can also mediate protective immunity against helminthes and ticks.[22,23]

Basophils, in contrast to mast cells, have long been thought not to synthesize arachidonic acid metabolites other than leukotriene C4 and platelet activating factor (PAF).[24–28] However, a recent flow cytometric study showed that hematopoietic-type prostaglandin D synthase (H-PGDS), which isomerizes PGH2 into PGD2,[29] is localized intracellularly in human basophils,[3] although a detailed examination of PGD2 generation from basophils was not performed. In the present study, we aimed to clarify whether basophils are capable of producing PGs. Our study showed that both mouse and human basophils secrete PGD2 and/or PGE2 after the cross-linking of FcεRI receptors, implying a possible contribution of basophils to inflammatory and/or immune reactions by functioning as a productive source of PGD2 and PGE2.

Materials and Methods

Animals

C57BL/6 and BALB/c mice (6 to 10 weeks old) were purchased from Sankyo Labo Service (Tokyo, Japan). FcRγ-chain$^{-/-}$C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All experiments in this study were performed according to the Guideline for Animal Use and Experimentation as set out by Tokyo Medical and Dental University.

Cell Preparation and Culture

Bone marrow-derived basophils (BMBAs) were prepared by culturing bone marrow cells in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and 10 ng/mL recombinant IL-3 (R&D Systems, Minneapolis, MN) for 10 days, followed by isolation of CD49b$^+$ cells with the use of a magnetically activated cell sorter system with biotinylated anti-CD49b and streptavidin microbeads (Miltenyi Biotec, Auburn, CA).

Bone marrow-derived mast cells (BMMCs) were obtained by culturing bone marrow cells in the presence of 10 ng/mL rIL-3 for 30 days (10, 20, and 30 days for Western blotting), followed by magnetic sorting of c-kit$^+$ cells with biotinylated anti-c-kit and streptavidin microbeads.

Primary basophils were prepared by enrichment of CD49b$^+$ cells from freshly isolated bone marrow cells with the use of the magnetically activated cell sorter system described above. CD49b$^+$ cells include ~20% basophils, as determined by flow cytometric analysis of CD49b and CD123.

Antibodies

Horseradish peroxidase–conjugated goat anti-rat IgG and rabbit anti-Actin IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Isotype-matched control antibodies (Abs; rat IgG2aκ and mouse IgG1κ), purified rat anti-mouse CD16/CD32 (2.4G2), and biotinylated monoclonal Abs (mAbs) specific for CD49b (Dx5) and c-kit (2B8) were from BD Pharmingen (San Diego, CA). Fluorescein isothiocyanate–conjugated anti-CD49b (Dx5) and phosphatidylethanolamin–anti-mouse CD123 (IL-3Rα) were purchased from eBioscience (San Diego, CA). Horseradish peroxidase–conjugated goat anti-rabbit IgG was from Dako (Glostrup, Denmark), and goat anti-human IgE (epsilon) IgG was from Invitrogen Life Technology (Carlsbad, CA). Anti-mMCP-8 (mouse mast cell protease 8) mAb (TUG8) was established by the first author (T.U.). Anti-H-PGDS mAb (7H4) and anti-mPGES (membrane-bound PGE synthase) mAb (6C6)[30] were established in Osaka Bioscience Institute (Osaka, Japan).

Florescent-Ab Conjugation

Anti-mMCP-8 mAb[31] was conjugated with Alexa 488, and both anti-H-PGDS mAb and anti-mPGES mAb[30] were conjugated with Alexa 647 by using the Alexa Fluor Monoclonal Antibody Labeling kit (Invitrogen).

Stimulation of Basophils and Mast Cells

For IgE-mediated stimulation, cells were sensitized with 0.5 μg/mL anti-TNP (trinitrophenyl)-IgE (IGEL-b4) in the presence of 10 ng/mL IL-3, then washed, and stimulated with 20 ng/mL TNP-OVA (Bioresearch Technologies, Novato, CA) or OVA (Sigma-Aldrich, St Louis, MO) for 30 minutes. For IgG-mediated stimulation, cells were stimulated with an immune complex composed of 20 μg/mL anti-TNP IgG (TIB-191, a generous gift from Dr. Karasuyama, Tokyo Medical and Dental University) and 40 μg/mL TNP-OVA or OVA for 30 minutes.

Isolation and Stimulation of Human Blood Basophils

Candidate blood donors were initially screened from healthy volunteers on the basis of the response of their basophils in a basophil activation test (Allergenicity kit; Beckman Coulter, Inc., Fullerton, CA), which assessed CD203c expression in response to IgE stimulation. Blood basophils were separated by Ficoll gradient centrifugation from venous whole blood anticoagulated with EDTA, followed by negative selection with the use of a Human Basophil Isolation Kit (Miltenyi Biotec). Basophil purity was >98%. Basophils were primed with 10 ng/mL human IL-3 (Sigma-Aldrich) for 6 hours, then washed, and stimulated with anti-human IgE (1 μg/mL) for 30 minutes.

Measurement of PGD2 and PGE2

The cells were harvested after stimulation. Culture supernatant fluids were collected, and PGs were purified as described previously. In brief, acidified culture supernatant fluids were added to solid phase extraction cartridges (C-18; Cayman Chemical, Ann Arbor, MI), followed by elution with ethyl acetate containing 1% methanol.[4] The concentrations of PGD2 and PGE2 were measured by using the ProstaglandinD2-Mox enzyme immunosorbent assay (EIA) kit (Cayman Chemical) and the Prostaglandin E2 EIA kit (Cayman Chemical), respectively.

High-Performance Liquid Chromatography with Tandem Mass Spectrometry

Negative ionspray tandem mass spectrometric measurements of PGs were conducted with an API 3200 (Applied Biosystems, Foster City, CA) equipped with NANOSPACE SI-2 system (SHISEIDO, Tokyo, Japan). Inertsil ODS-3 HPLC columns (GL Science, Torrance, CA) were used for high-performance liquid chromatography (HPLC) separations with a mobile phase consisting of 10% to 100% acetonitrile gradients 0.01 acetic acid at a flow rate of 200 μL/minute.

Immunoblotting

Cells were lysed in lysis buffer containing 0.5% Triton X-100 (MP Biochemical, Solon, OH) and a protease inhibitor cocktail (Sigma-Aldrich). Total cell lysates were subjected to SDS-PAGE (10% polyacrylamide), followed by immunoblotting with the indicated antibodies and horseradish peroxidase–conjugated secondary Abs. Proteins were visualized with the ECL Plus Western Blotting System (GE Healthcare, Buckinghamshire, UK).

Cytospin and Flow Cytometric Analyses

Cytospin preparations were fixed in methanol and treated with blocking solution containing 10% normal goat serum, 0.01% Triton X-100, and 0.1% NaN3 to prevent nonspecific Ab binding. The preparations were incubated with a combination of Alexa 488-conjugated anti-mMCP-8 mAb and Alexa 647-conjugated anti-H-PGDS mAb or Alexa 647-conjugated anti-mPGES1 mAb and were examined under a confocal laser scanning microscope (LSM510; Carl Zeiss, Oberkochen, Germany). For flow cytometric analyses, single-cell suspensions were treated with 2.4G2 and normal rat serum and were subsequently stained with the indicated Abs. Cells were analyzed with a FACSCaliber (BD Biosciences, Mountain View, CA).

Semiquantitative RT-PCR and Real-Time PCR Analysis

Total RNA was prepared from cells, and first-strand cDNA was synthesized with reverse transcription with oligo-dT primers. For semiquantitative RT-PCR, PCR was performed with a fivefold serially diluted cDNA template at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds for 35 cycles, followed by further extension at 72°C for 5 minutes. For real-time PCR, the expression of each gene was measured relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with the use of SYBR Green dye (Applied Biosystems) with real-time PCR systems. The following primers (forward and reverse, respectively) were used for both PCR reactions: for *Hpgds*, 5′-ATCCACCAGAGCCTCGCAATAG-3′ and 5′-TCATCCAGCGTGTCCACCA-3′; for *Ptgds*, 5′-GACACAGTGCAGCCCAACTTTC-3′ and 5′-GGGCTACCACTGTCTTGCACATA-3′; for *Ptges1*, 5′-GGATGCGCTGAAACGTGGA-3′ and 5′-CAGGAATGAGTACACGAAGCC-3′; for *Ptges2*, 5′-CTCATCAGCAAGCGCCTCAA-3′ and 5′-GGTCTTTACCCACGGCTGTCA-3′; for *Ptges3,* 5′-ATCACATGGGTGGTGATGAGGA-3′ and 5′-AGGCGATGACAACAGCCCTTAC-3′; and for *GAPDH*, 5′-TTCACCACCATGGAGAAGGCCG-3′ and 5′-GGCATGGACTGTGGTCATGA-3′.

Results

BMBAs Release Both PGD2 and PGE2 in Response to IgE + Ag, but Not IgG + Ag

To begin to assess whether basophils release PGD2, BMBAs were prepared from mice and tested for their ability to generate PGD2. BMBAs primed with anti-TNP-IgE secreted PGD2 in response to TNP-OVA, indicating that BMBAs produce PGD2 after IgE-mediated stimulation (Figure 1A). Moreover, to our surprise, BMBAs also generated PGE2 in response to Ag-specific IgE + Ag, albeit to a lesser extent than PGD2 (Figure 1B). Basophils have been reported to release PAF, an arachidonic acid metabolite, on stimulation of IgG receptors, thereby eliciting systemic anaphylaxis.[19] Thus, we next explored whether BMBAs produce PGD2 and/or PGE2 via IgG-mediated stimulation. However, neither PGD2 nor PGE2 was released after stimulation of BMBAs with an immune complex of anti-TNP-IgG and TNP-OVA (Figure 1, C and D).

FcεRI Is Involved in IgE-Mediated PGD2/E2 Secretion

To confirm the involvement of the high-affinity IgE receptor, FcεRI, in PG secretion, we analyzed PGD2 and PGE2 generation in BMBAs prepared from FcRγ-chain$^{-/-}$ mice lacking FcεRI expression. Basophils from FcRγ-chain$^{-/-}$ mice were determined by their expression of CD49b and CD123 instead of CD49b and FcεRI, as previously reported.[32] We first confirmed that BMBAs differentiated normally even in the absence of the FcRγ-chain (Figure 1E). The PGD2 and PGE2 secretion observed from BMBAs of wild-type C57BL/6 mice was completely abrogated in BMBAs of FcRγ-chain$^{-/-}$ mice (Figure 1, F and G). These data indicated that PGD2 and PGE2 release from BMBAs depend on FcεRI-mediated, but not CD23-mediated, signals.

Primary Basophils also Produce both PGD2 and PGE2 in Response to FcεRI-Mediated Stimulation

We next attempted to verify whether primary basophils are also capable of producing PGD2 and/or PGE2. Primary basophils, that is, CD49b$^+$ cells freshly isolated from bone marrow, were stimulated with anti-TNP IgE and TNP-OVA. We first measured PGD2 and PGE2 in their supernatant fluid with the use of EIA as described earlier. Primary basophils produced more or less the same amount of PGD2 as that from BMBAs (Figure 2). However, PGE2 secretion from primary basophils was higher than that from BMBAs. The ability of primary basophils to produce PGD2 and PGE2 was further confirmed by analysis of PGs with the use of HPLC with tandem mass spectrometry. Both PGD2 and PGE2 were detected in basophil supernatant fluids by HPLC-tandem mass spectrometry analysis (Figure 3). This result clearly shows that basophils produce both PGD2 and PGE2. Primary basophils appear to be more potent for PGE2 production than BMBAs.

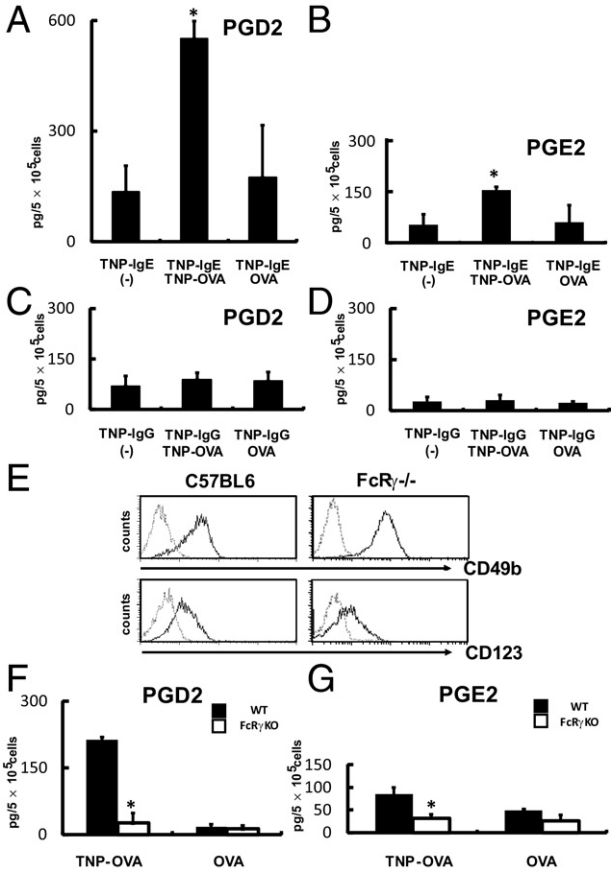

Figure 1. BMBAs secrete both PGD2 and PGE2 after cross-linking of FcεRI receptors. **A** and **B:** BMBAs prepared from bone marrow cells of BALB/c mice were sensitized with 0.5 μg/mL anti-TNP-IgE overnight, followed by stimulation with 20 ng/mL TNP-OVA or OVA for 30 minutes. The concentration of PGD2 and PGE2 in the supernatant fluids was determined with EIA. BMBAs produced both PGD2 and PGE2 in response to IgE-mediated stimulation. **C** and **D:** BMBAs were stimulated with 20 μg/mL anti-TNP-IgG and 40 μg/mL TNP-OVA for 30 minutes. **E:** BMBAs from C57BL/6 FcRγ-chain$^{-/-}$ mice were identified as CD49b$^+$ CD123$^+$ cells by flow cytometric analysis. Differentiation of basophils was not affected by the absence of the FcRγ chain. **F** and **G:** Neither PGD2 nor PGE2 was produced by BMBAs prepared from C57BL/6 FcRγ-chain$^{-/-}$ mice after *in vitro* stimulation with IgE+ Ag. $^*P < 0.05$ (Student's *t*-test). Error bars indicate standard deviation.

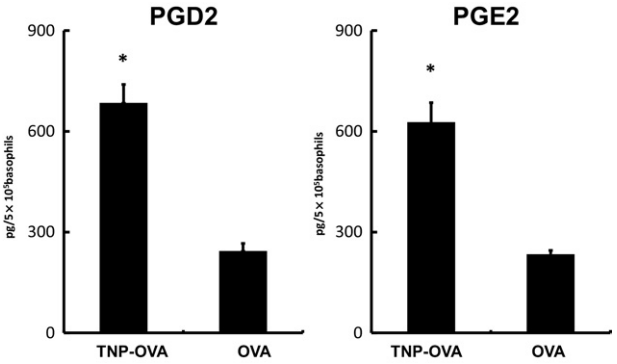

Figure 2. PGD2 and PGE2 production from primary basophils. Primary basophils (CD49b$^+$ cells freshly isolated from bone marrow cells) were sensitized with 0.5 μg/mL anti-TNP-IgE, and then stimulated with 20 ng/mL TNP-OVA or OVA for 30 minutes. The concentration of PGD2 and PGE2 in the supernatant fluids was determined with EIA. Primary basophils secreted comparable levels of PGE2 and PGD2. $^*P < 0.05$, compared with OVA stimulation. (Student's *t*-test). Error bars indicate standard deviation.

PGD2 and PGE2 Production by BMMCs

Although mast cells are widely known to produce PGD2,[2,33] PGE2 production by mast cells has not been well studied. We next assessed the generation of both PGE2 and PGD2 by BMMCs. BMMCs prepared from BALB/c mice released ~1.5 times as much PGD2 as primary basophils in response to TNP-OVA (Figure 4) but, in contrast, did not secrete as much PGE2 as primary basophils.

Basophils Express H-PGDS and PGES

We next analyzed the expression of PGD2 and PGE2 synthesizing enzymes in murine basophils with the use of RT-

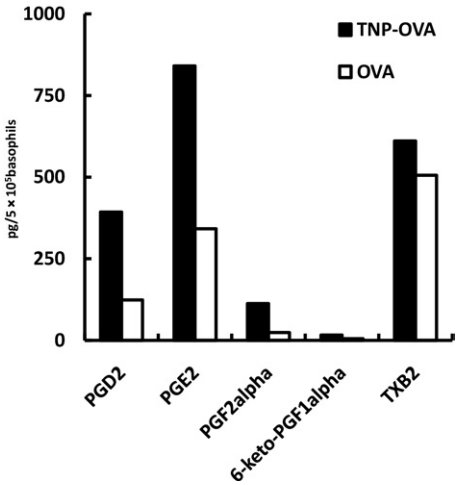

Figure 3. HPLC-tandem mass spectrometry analysis of PGs produced by primary basophils. Primary basophils sensitized with anti-TNP-IgE overnight were stimulated with TNP-OVA or OVA. The level of PGs in the supernatant fluids was determined with HPLC-tandem mass spectrometry. Primary basophils secreted both PGD2 and PGE2 but not PGF1/2 or TXB2. TXB2, thromboxan B2.

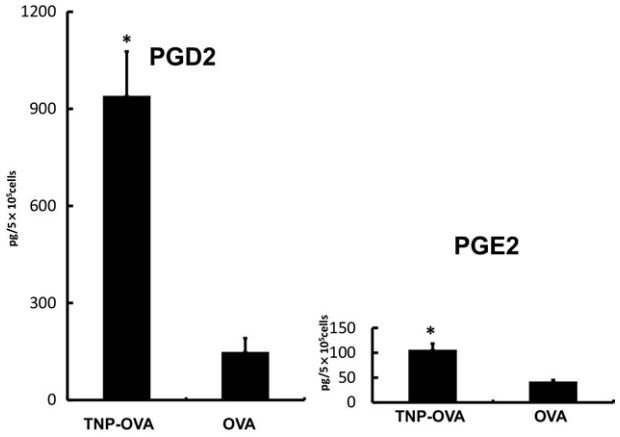

Figure 4. PGD2 and PGE2 production by BMMCs. BMMCs prepared from BALB/c mice sensitized with 0.5 μg/mL anti-TNP-IgE released a significant amount of PGD2 in response to 20 ng/mL TNP-OVA but a low level of PGE2. $^{*}P < 0.05$, compared with OVA stimulation (Student's *t*-test). Error bars indicate standard deviation.

PCR and Western blotting. PGD2 is synthesized by isomerization of PGH2 through PGDS.[29] PGDS is classified into two types, H-PGDS and lipocalin-type PGD synthase (L-PGDS).[29,34] PGE2 is synthesized by isomerization of PGH2 through PGES. PGES is classified into three types, glutathione-dependent membrane-bound PGES (mPGES1), glutathione-independent membrane-bound PGES (mPGES2), and cytosolic PGES (cPGES).[35–37] *Hpgds* transcripts that encode H-PGDS were readily detected in both BMBAs and BMMCs by RT-PCR. In contrast, *Ptgds* transcripts that encode L-PGDS were only detected in BMMCs (Figure 5, A and B). We analyzed H-PGDS protein expression in BMBAs and BMMCs at various stages of their development by immunoblotting. H-PGDS expression was relatively low in BMBAs ($CD49b^{+}$, $c\text{-}kit^{-}$) and in 10-day–cultured BMMCs ($CD49^{-}$, $c\text{-}kit^{+}$), but was increased in 20-day–cultured and 30-day–cultured BMMCs (Figure 6A), indicating that H-PGDS activity in BMMCs increased along with their differentiation. *Ptges1*, *Ptges2*, and *Ptges3* transcripts, which encode mPGES1, mPGES2, and cPGES, respectively, were all detected in BMBAs as well as in BMMCs (Figure 5, A and C).

Furthermore, bone marrow cells expressing mMCP-8, a specific marker for basophils,[31] were positive for both H-PGDS and mPGES1 by immunohistochemical analysis (Figure 6B). These results show that basophils express H-PGDS as well as PGES, thereby producing both PGD2 and PGE2.

Human Blood Basophils also Produce PGD2 but Not PGE2

A prior flow cytometric study reported the presence of H-PGDS in human blood basophils.[3] We therefore examined whether human basophils produce PGD2 and/or PGE2 after IgE-mediated stimulation. We primed human blood basophils isolated from several healthy donors with IL-3 and then stimulated them with anti-human IgE Ab for 30 minutes. Human basophils secreted PGD2 on stimulation (Figure 7). Unlike murine basophils, however, PGE2 generation from human basophils was barely detectable (data not shown).

Discussion

It has long been believed that basophils do not synthesize arachidonic acid metabolites except for leukotriene C4 and PAF.[27,28] However, in the present study, we demonstrated for the first time that mouse basophils produce both PGD2 and PGE2 after aggregation of FcεRI receptors. In addition, we found that human basophils are also capable of producing PGD2 after IgE-mediated stimulation.

The expression of the two types of PGDS, H- and L-PGDS, varies according to cell type.[29,34] Mast cells, antigen-presenting cells, and a small population of Th2 cells express H-PGDS.[2–4,33] L-PGDS is expressed in meningeal cells, epithelial cells of the choroid plexus, and oligodendrocytes in the brain.[38] In our study, the gene encoding H-PGDS, but not that encoding L-PGDS, was expressed in BMBAs. H-PGDS proteins were detected in BMBAs as well as in primary basophils. Thus, H-PGDS appears to be a major enzyme involved in PGD2 generation in basophils. BMMCs released a greater amount of PGD2 than BMBAs (Figures 1A and 2). The higher production of PGD2 by BMMCs relative to BMBAs

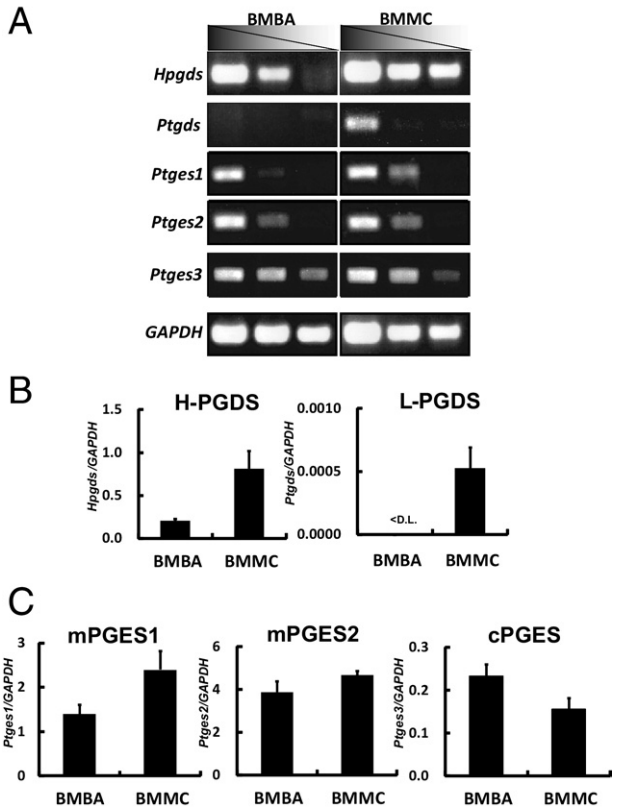

Figure 5. Expression of PG synthase (PGS) transcripts in BMBAs and BMMCs. The expression of the indicated genes in BMBAs and BMMCs was determined by quantitative RT-PCR analysis of total cellular RNA. **A:** Gel electrophoresis of the PCR products. The PCR templates were fivefold serially diluted. **B** and **C:** The expression of each gene was measured relative to GAPDH with the use of SYBR Green dye with real-time PCR systems. Error bars indicate standard deviation.

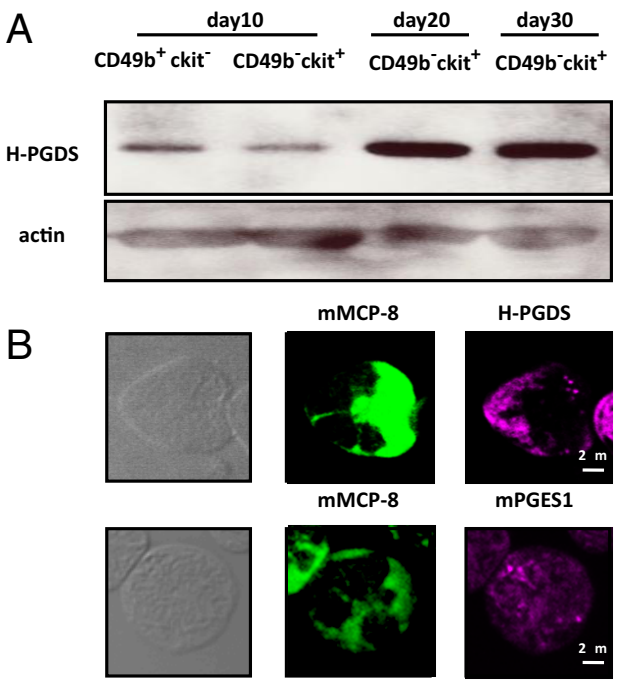

Figure 6. Immunoblotting and immunohistochemical analyses of PG synthase proteins in basophils and mast cells. **A:** Cell lysates were prepared from BMBAs (CD49b$^+$ fraction of bone marrow cells cultured with rIL-3 for 10 days) and BMMCs at various stages of their development (c-kit$^+$ fractions of bone marrow cells cultured with rIL-3 for 10, 20, or 30 days) and were subjected to immunoblotting with the indicated antibodies. Actin was used as a loading control. **B:** Cytospin slides prepared from bone marrow CD49b$^+$ cells (primary basophils) were stained with Alexa 488-conjugated anti-mMCP-8 mAb and Alexa 647-conjugated anti-H-PGDS mAb or Alexa 647-conjugated anti-mPGES1 mAb. Intracellular H-PGDS and mPGES1 proteins were detected in mMCP-8 (+) cells. Phase contrast images are at left.

was consistent with the findings that levels of H-PGDS and L-PGDS expression were higher in BMMCs than in BMBAs (Figure 5, A and B).

The class of PGES expressed also varies according to cell type.[35–37] Expression of mPGES1 is markedly induced by pro-inflammatory stimulation in various tissues.[35] PGES1 is involved in the COX-2–mediated PGE2-biosynthetic pathway[35]; mPGES2 is abundant in brain, heart, skeletal muscle, kidney, and liver[37]; cPGES is distributed ubiquitously in the cytosol of various cells[36]; and COX-1 contributes to PGE2 generation by PGES2 and cPGES.[36,39,40] We immunohistochemically confirmed the presence of mPGES1 in primary basophils, but we were unable to assess the protein expression of the other PGES enzymes, because antibodies against mPGES2 and cPGES were not available in our laboratory. Nevertheless, transcripts encoding mPGES1, mPGES2, and cPGES were all detected in BMBAs. The subtype of PGES that is actually involved in PGE2 generation in basophils is uncertain. However, a COX-1–coupled PGES, such as mPGES2 or cPGES, may mediate immediate PGE2 production. In mast cells, COX-1, but not COX-2, is responsible for immediate PGD2 production after cell stimulation with IgE + Ag.[41]

In contrast to the high production of PGD2 from BMMCs, PGE2 generation from BMMCs was low, despite that transcripts of all of the genes that encode PGES were detected. IgE-mediated signals may not provide an efficient stimulus for induction of mast cell-derived PGE2 production.

Basophils promote the initiation of IgE-CAI associated with marked eosinophil infiltration.[18] Treatment with a basophil-depleting Abs prevents the development of IgE-CAI,[42] indicating the essential role of basophils in this reaction. Our prior study showed that the number of infiltrative basophils in skin lesions of IgE-CAI was higher than that of dermal mast cells (19.4 cells/mm^2 versus 8.2 cells/mm^2).[31] Thus, basophils may be equally or even more important than mast cells as sources of PGD2 production in IgE-CAI. PGD2 is one of the important mediators responsible for the development of IgE-CAI as evidenced by unpublished observation (Y. Matsushima) that the administration of an antagonist against the PGD2 receptor CRTH2 ameliorated skin inflammation.

Although stimulation of the IgG receptors induces PAF release from basophils,[19] in the current study these stimulations did not result in PGD2/E2 synthesis by basophils. Although we have not been able to assess actual differences in the signals mediated by IgE and IgG in basophils, FcγR signals, unlike FcεRI signals, may preferentially activate the PAF synthesis and the 5-liooxygenase pathway rather than the COX-PG synthesis pathway.

In this study, we confirmed that human basophils are also capable of producing PGD2. This result was in striking contrast to a prior study which reported that basophils do not produce PGs.[27] This discrepancy could be due to differences in preparation of the basophil samples or in the assay systems or cell priming methods. The previous study isolated basophils with the use of the method by MacGlanshan et al,[43] and cell purity ranged from 64% to 92%. Some of these cells might have been non-releasing basophils, because basophils from 10% to 20% of human peripheral blood donors show defects in histamine release.[44] There have also been previous attempts to detect PG expression with the use of radioimmunoassay and gas chromatography mass spectrometry which gave negative results.[27] In the present study, we only prepared highly enriched basophils from volunteers who had histamine-releasing basophils on the basis of the results of a

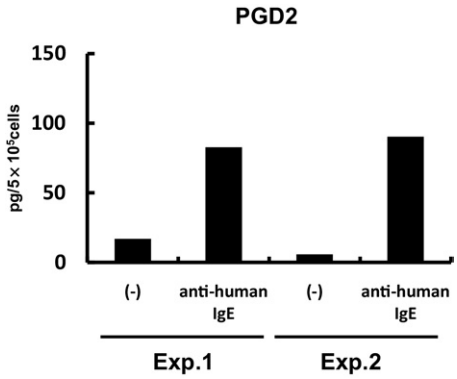

Figure 7. Human blood basophils also secrete PGD2 in response to IgE-mediated stimulation. Human blood basophils obtained from the venous blood of healthy donors were primed with 10 ng/mL human IL-3 and then stimulated with 1 μg/mL anti-human IgE Ab for 30 minutes. The concentration of PGD2 and PGE2 in the supernatant fluids was determined with EIA. Human blood basophils produced PGD2 after IgE-mediated stimulation. The results of two separate experiments (exp. 1 and exp. 2) are shown.

basophil activation test (see *Materials and Methods*). Furthermore, these basophils were primed with IL-3, because IL-3 has been shown to induce phosphorylation of phospholipase A_2.[28] In addition, in our study, PGs were detected with EIA, which is a more sensitive detection system than radioimmunoassay and gas chromatography mass spectrometry. These combined approaches may have been the reason why our study, in contrast to earlier studies, could successfully detect PG expression in basophils.

Unlike mouse basophils, human basophils did not generate a large amount of PGE2. More intense stimulation than anti-human IgE might be required for the induction of PGE2 release. Alternatively, arachidonic acid metabolite cascades in human basophils may be different from those of mice. Thus, human basophils may favor the H-PGDS pathway over the PGES pathway.

IgE-CAI in mice appears to share morphologic similarities with some of the skin lesions of atopic dermatitis, long-lasting urticaria, and/or prurigo reaction in humans. Our prior study suggested that there was a possible involvement of the PGD2-CRTH2 interaction in these diseases.[45] Most notably, the skin lesions found in these diseases are histologically characterized by a marked basophil infiltration.[46] Thus, basophil-derived PGD2 may at least partly contribute to the pathogenesis of these inflammatory skin reactions.

In summary, the present study showed an overlooked function of basophils: the production of PGD2 and PGE2 through FcεRI-mediated signaling. Basophils may be involved in IgE-mediated allergic inflammation and immune responses via the release of PGD2 and/or PGE2.

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