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Analysis of Two Major Intracellular Phospholipases A_2 (PLA_2) in Mast Cells Reveals Crucial Contribution of Cytosolic $PLA_2\alpha$, Not Ca^{2+}-independent $PLA_2\beta$, to Lipid Mobilization in Proximal Mast Cells and Distal Fibroblasts*[S]

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Noriko Ueno,[a] Yoshitaka Taketomi,[a,b] Kei Yamamoto,[a] Tetsuya Hirabayashi,[a] Daisuke Kamei,[b] Yoshihiro Kita,[c,d] Takao Shimizu,[c] Koei Shinzawa,[e] Yoshihide Tsujimoto,[e] Kazutaka Ikeda,[f,g,h1] Ryo Taguchi,[f,g,i1] and Makoto Murakami[a,j2]

From the [a]Lipid Metabolism Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 256-8506, the [b]Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, the Departments of [c]Biochemistry and Molecular Biology, [d]Lipidomics, and [f]Metabolome, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, the [e]Laboratory of Molecular Genetics, Department of Medical Genetics, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, the [g]Core Research for Evolutional Science and Technology and [j]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, the [h]Institute for Advanced Biosciences, Keio University, 246-2 Mizukami, Kakuganji, Tsuruoka, Yamagata, 997-0052, and the [i]College of Life and Health Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan

Background: Mast cells express $cPLA_2\alpha$ and $iPLA_2\beta$.
Results: Knock-out of $cPLA_2\alpha$, not $iPLA_2\beta$, hampers arachidonic acid mobilization in mast cells and adjacent fibroblasts.
Conclusion: Mast cell $cPLA_2\alpha$ is coupled with stromal synthesis of anti-allergic PGE_2, whereas $iPLA_2\beta$ is dispensable for mast cell function.
Significance: The $cPLA_2\alpha$-dependent transcellular PGE_2 synthesis opens new insight into the lipid biochemistry and mast cell biology fields.

Mast cells release a variety of mediators, including arachidonic acid (AA) metabolites, to regulate allergy, inflammation, and host defense, and their differentiation and maturation within extravascular microenvironments depend on the stromal cytokine stem cell factor. Mouse mast cells express two major intracellular phospholipases A_2 (PLA_2s), namely group IVA cytosolic PLA_2 ($cPLA_2\alpha$) and group VIA Ca^{2+}-independent PLA_2 ($iPLA_2\beta$), and the role of $cPLA_2\alpha$ in eicosanoid synthesis by mast cells has been well documented. Lipidomic analyses of mouse bone marrow-derived mast cells (BMMCs) lacking $cPLA_2\alpha$ (*Pla2g4a*$^{-/-}$) or $iPLA_2\beta$ (*Pla2g6*$^{-/-}$) revealed that phospholipids with AA were selectively hydrolyzed by $cPLA_2\alpha$, not by $iPLA_2\beta$, during FcεRI-mediated activation and even during fibroblast-dependent maturation. Neither FcεRI-dependent effector functions nor maturation-driven phospholipid remodeling was impaired in *Pla2g6*$^{-/-}$ BMMCs. Although BMMCs did not produce prostaglandin E_2 (PGE_2), the AA released by $cPLA_2\alpha$ from BMMCs during maturation was converted to PGE_2 by microsomal PGE synthase-1 (mPGES-1) in cocultured fibroblasts, and accordingly, *Pla2g4a*$^{-/-}$ BMMCs promoted microenvironmental PGE_2 synthesis less efficiently than wild-type BMMCs both *in vitro* and *in vivo*. Mice deficient in mPGES-1 (*Ptges*$^{-/-}$) had an augmented local anaphylactic response. These results suggest that $cPLA_2\alpha$ in mast cells is functionally coupled, through the AA transfer mechanism, with stromal mPGES-1 to provide anti-anaphylactic PGE_2. Although $iPLA_2\beta$ is partially responsible for PGE_2 production by macrophages and dendritic cells, it is dispensable for mast cell maturation and function.

Mast cells are important effector cells for IgE-associated allergic reactions, such as anaphylaxis, rhinitis, and asthma, and represent an important source of various inflammatory mediators, including vasoactive amines, proteases, arachidonic acid (AA)[3] metabolites,

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[2] Supported by Precursory Research for Embryonic Science and Technology from the Japan Science and Technology Agency, The Toray Foundation, The Mitsubishi Science Foundation, The Terumo Life Science Foundation, and The Uehara Memorial Foundation. To whom correspondence should be addressed: Lipid Metabolism Project, The Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan. Tel.: 81-3-5316-3228; Fax: 81-3-5316-3125; E-mail: murakami-mk@igakuken.or.jp.

[3] The abbreviations used are: AA, arachidonic acid; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; $iPLA_2$, Ca^{2+}-independent PLA_2; $sPLA_2$, secreted PLA_2; BMMC, bone marrow-derived mast cell; CTMC, connective tissue mast cell; DNP, dinitrophenyl; ESI-MS, electrospray ion source-mass spectrometry; β-HEX, β-hexosaminidase; LT, leukotriene; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, prostaglandin; PNPLA, patatin-like phospholipase A domain-containing lipase; SCF, stem cell factor; LPC, lysophosphatidylcholine; CRAC, Ca^{2+} release-activated Ca^{2+}; EPA, eicosapentaenoic acid; OA, oleic acid; LA, linoleic acid; BEL, bromoenol lactone; BMDC, bone marrow-derived dendritic cell; Ag, antigen; PCA, passive cutaneous anaphylaxis.

cytokines, and chemokines. Mast cells orchestrate various aspects of the IgE-dependent and -independent immune responses not only through the release of these factors but also through cell-cell interaction by which they regulate the function of other cells. Mast cell precursors originating from bone marrow circulate in the blood and migrate into connective or mucous tissues, where they differentiate into mature mast cell phenotypes depending on tissue microenvironments (1–4). Stromal fibroblasts support the homing, growth, differentiation, and survival of mast cells by stem cell factor (SCF) and its receptor c-Kit as well as by other factors, including cytokines, chemokines, and adhesion molecules (5–8). Coculture of IL-3-maintained immature mouse bone marrow-derived mast cells (BMMCs) with fibroblasts is a useful *in vitro* system for analyzing certain aspects of the change to a mature connective tissue mast cell (CTMC)-like phenotype (9–14). Conversely, mast cells, though as yet unknown mechanisms, can affect the proliferation and functions of surrounding fibroblasts leading to collagen deposition and fibrosis, which are central features of chronic inflammation and tissue repair (15–21).

AA-derived eicosanoids (prostaglandins (PGs) and leukotrienes (LTs)) are produced in substantial amounts in tissues harboring T_{H2}-based allergic responses (22, 23). Activated mast cells produce LTB_4 and LTC_4, which play crucial roles in allergic responses by facilitating bronchoconstriction, vascular permeability, and inflammatory cell recruitment (23, 24). PGD_2 is a major prostanoid produced by activated mast cells (25, 26), and studies using mice deficient in the PGD receptor DP1 or DP2 (CRTH2) have revealed the roles of PGD_2 in promotion or prevention of allergic responses in distinct cellular contexts (27–30). In contrast, gene targeting of the PGE receptor EP3 (31) or the biosynthetic enzyme microsomal PGE_2 synthase (mPGES-1) (32) has demonstrated that PGE_2 acts as a negative modulator of asthmatic reactions and that the PGE_2-EP3 axis appears to be the long sought mechanism that explains the aspirin intolerance seen in asthma. Because mast cells produce PGE_2 only minimally, stromal cells (*e.g.* fibroblasts) that surround mast cells may supply PGE_2 to tissue microenvironments. However, how stromal PGE_2 synthesis is regulated in local tissues where mast cells also reside is not fully understood.

Biosynthesis of prostanoids is initiated by the release of AA from membrane phospholipids by phospholipase A_2 (PLA_2). PLA_2 is also considered to be important for the regulation of membrane remodeling. To date, more than 30 different PLA_2 enzymes have been identified in mammals and are subdivided into three major classes as follows: cytosolic PLA_2 ($cPLA_2$) and Ca^{2+}-independent PLA_2 ($iPLA_2$), which are high molecular weight intracellular PLA_2s with a catalytic serine, and secreted PLA_2 ($sPLA_2$), which represents a group of low molecular weight, Ca^{2+}-requiring enzymes with a His-Asp catalytic dyad (33, 34). Of the intracellular PLA_2s, $cPLA_2\alpha$ and $iPLA_2\beta$ are the "big two," whose functions and regulatory mechanisms have been well studied (reviewed in Ref. 34). In mast cells, group IVA $cPLA_2\alpha$ plays a critical role in PGD_2 and LTC_4 production after various stimuli such as FcϵRI cross-linking or cytokines (35, 36). The AA released by $cPLA_2\alpha$ at the perinuclear Golgi membrane is converted by the sequential action of cyclooxygenase (COX) and terminal PG synthases to prostanoids or 5-lipoxygenase and terminal LT synthases to LTs. Group V $sPLA_2$, in cooperation with $cPLA_2\alpha$, reportedly augments PGD_2 production by BMMCs in response to zymosan (37) and promotes PGE_2 production by neighboring fibroblasts through the transcellular pathway (38). Mast cells also express $iPLA_2\beta$, a prototypic $iPLA_2$ isoform, and the $iPLA_2$ inhibitor bromoenol lactone (BEL) can attenuate granule exocytosis by mast cells (39), leading to the suggestion that $iPLA_2\beta$ participates in the regulation of mast cell activation. Although $iPLA_2\beta$ has long been thought to play a role in phospholipid remodeling (40), recent studies using mice or cells that are devoid of $iPLA_2\beta$ as a result of gene targeting or siRNA knockdown have defined its roles in various signaling events, such as Ca^{2+} release-activated Ca^{2+} (CRAC) channel opening (41), lipid mediator generation (42, 43), exocytosis (44), cytokine secretion (45), cell migration (46), vascular contractility (47), apoptosis (48, 49), cancer (50), and neuronal degeneration (51, 52). Because of its roles in Ca^{2+} gating and lipid mediator generation, the sequential action of $iPLA_2\beta$ and $cPLA_2\alpha$ for the full operation of AA release has been proposed in vascular cells (42). However, the roles of $iPLA_2\beta$ in mast cells have not yet been fully elucidated.

In this study, using BMMCs null for $cPLA_2\alpha$, $iPLA_2\beta$, group V $sPLA_2$, or mPGES-1, in combination with lipidomic mass spectrometry (MS), we provide the following evidence: (i) $cPLA_2\alpha$ plays a fundamental role in AA-selective release from BMMCs during FcϵRI-mediated activation and even during fibroblast-dependent maturation; (ii) $iPLA_2\beta$ minimally mobilizes phospholipids in mast cells during these processes; (iii) the AA selectively released by $cPLA_2\alpha$ from BMMCs during maturation is transferred to adjacent fibroblasts to be metabolized to PGE_2 by mPGES-1, whereas group V $sPLA_2$ in BMMCs fails to participate in this process; and (iv) mPGES-1-driven PGE_2 has a negative regulatory role in the allergen-triggered anaphylactic response. Overall, our findings underscore the importance of mast cell $cPLA_2\alpha$ not only in the proximal production of mast cell-derived pro-allergic eicosanoids (PGD_2 and LTC_4) but also in the distal production of an anti-allergic eicosanoid (PGE_2) by fibroblasts through the cell-to-cell AA transfer mechanism. Contrary to our expectation, however, $iPLA_2\beta$ was found to play no role in effector functions or phospholipid membrane remodeling in mast cells.

EXPERIMENTAL PROCEDURES

Mice—C57BL/6 mice were purchased from Japan SLC, Inc. Mice deficient in $cPLA_2\alpha$ (*Pla2g4a*$^{-/-}$) (36), $iPLA_2\beta$ (*Pla2g6*$^{-/-}$) (52), group V $sPLA_2$ (*Pla2g5*$^{-/-}$) (37), and mPGES-1 (*Ptges*$^{-/-}$) (53), all on a C57BL/6 background, were described previously. We used 8–12-week-old mice for all experiments. The genotypes of individual knock-out mice and their littermates were confirmed by PCR analysis of genomic DNA obtained by tail biopsy. All procedures involving animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees of the Tokyo Metropolitan Institute of Medical Science and Showa University, in accordance with the Standards Relating to the Care and Management of Experimental Animals in Japan.

Culture and Activation of BMMCs, Macrophages, and Dendritic Cells—To prepare BMMCs, bone marrow cells were obtained from femurs and tibias of mice and cultured in IL-3-containing BMMC complete medium consisting of DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 100 mM nonessential amino acids, and an optimal amount of recombinant mouse IL-3 that had been produced by the baculovirus/insect cell system, as described previously (11–14). After 4–5 weeks of culture, the majority of floating cells was confirmed to be immature BMMCs as assessed by Alcian blue-positive and safranin O-negative staining of their granules.

Maturation of immature BMMCs toward CTMC-like cells was described previously in detail (11–14). Briefly, 5×10^6 BMMCs were seeded on a subconfluent Swiss 3T3 fibroblast monolayer in 100-mm culture dishes and cocultured for up to 4 days in the presence of the baculovirus/insect cell-produced soluble form of SCF with replacement of the medium every 2 days. The cells were trypsinized and replated, and nonadherent cells (>97% being mast cells) were collected and used for analyses. Maturation of BMMCs into CTMC-like cells was verified by staining of their granules with Alcian blue and counterstaining with safranin O.

BMMCs (10^7 cells/ml) were incubated for 1 h in culture medium containing 10 μg/ml anti-dinitrophenyl (DNP) IgE (Sigma) and then treated for appropriate periods with various concentrations of DNP-conjugated albumin as an antigen (Ag) (Sigma) in culture medium. The percentage release of β-hexosaminidase (β-HEX), a degranulation marker, was evaluated as described previously (11). Aliquots of the supernatants were subjected to enzyme immunoassays for lipid mediators (PGD_2, PGE_2, or LTC_4) (Cayman Chemicals) and IL-6 (eBioscience). As required for the experiments, the cells were activated in the presence of (*S*)- and (*R*)-BEL (Cayman Chemicals), which preferentially inhibit iPLA$_2\beta$ and iPLA$_2\gamma$, respectively (53, 54).

Bone marrow-derived macrophages (BMMΦs) and dendritic cells (BMDCs) were prepared by culture of mouse bone marrow cells in medium supplemented for 3 days with 50 ng/ml human macrophage colony-stimulating factor (Kyowa Kirin) or for 9 days with 10 ng/mouse GM-CSF (PeproTech), respectively. BMMΦs and BMDCs were stimulated for 3 h with 100 μg/ml zymosan (Sigma) and for 24 h with 100 ng/ml LPS (Sigma). PGE_2 levels in culture supernatants were measured by enzyme immunoassay as above.

Measurement of Intracellular Ca^{2+} Levels—IgE-sensitized BMMCs on coverslips were loaded for 60 min with the fluorescent Ca^{2+} indicator fura-2/AM (5 μM) (Invitrogen) in Tyrode/HEPES buffer, pH 7.4, containing 2.5 mM probenecid, 0.04% (v/v) pluronic acid, and 1% serum. Then the cells were washed and stimulated with Ag. Fura-2 fluorescence images were obtained using an ARGUS-50 image analyzer (Hamamatsu Photonics, Shizuoka, Japan) with excitation at 340 nm (F_{340}) and 380 nm (F_{380}) at 5-s intervals. The ratio (F_{340}/F_{380}) was calculated using ImageJ software (National Institutes of Health, Bethesda).

RT-PCR—Total RNA was extracted from BMMCs with TRIzol reagent (Invitrogen). First strand cDNA synthesis was carried out using a SuperScript III reverse transcriptase kit (Invitrogen). Total RNA (5 μg) was used in reactions primed with oligo(dT) (12–18-mer) primer (Invitrogen) to obtain cDNA. Then 1 μl of the synthesized cDNA was used as the template for the mRNA amplification reactions. The PCR amplification was performed with *exTaq* polymerase (Takara Biomedicals) using a GeneAmp PCR System 9600 (Applied Biosystems). The RT-PCR products were analyzed on a 1.5% agarose gel and visualized using ethidium bromide staining. The primer pairs used were as follows: mouse cPLA$_2\alpha$, 5′-ccactttgttctggccaaca-3′ and 5′-agggaaacagagcaacgaga-3′; mouse cPLA$_2\beta$, 5′-agccccatgaacagaaactc-3′ and 5′-caaagagctcggaggagatg-3′; mouse cPLA$_2\gamma$, 5′-agaacttggccagttggatg-3′ and 5′-gcactccttcttccacttgc-3′; mouse cPLA$_2\delta$, 5′-cggctgatgaagagacttcc-3′ and 5′-ctggtggaatggcctgccagtcag-3′; mouse cPLA$_2\epsilon$, 5′-ctgcatgaggatgaggtaccg-3′ and 5′-cctctcgccatttgtagagc-3′; mouse cPLA$_2\zeta$, 5′-gaagaacgtcctggagcttg-3′ and 5′-gtgagccaccaggacaccattgg-3′; mouse iPLA$_2\beta$, 5′-catgagtacaatcaggacctg-3′ and 5′-caatctgaagtactggatgccga-3′; and mouse mast cell protease (mMCP-4)-4, 5′-tgagagagggttcacagctac-3′ and 5′-tcacagagggagtctctttgg-3′. The PCR thermal conditions were as follows: 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s (for cPLA$_2$s); 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 40 s (for iPLA$_2\beta$), and 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s (for mMCP-4). RT-PCR for mouse GAPDH was performed as described previously (14). After 28 cycles of amplification, the PCR products were run on 1% agarose gels with ethidium bromide. Quantitative RT-PCR was carried out using a Power SYBR Green PCR system (Applied Biosystems) or a TaqMan Gene Expression System (Applied Biosystems) on the ABI7700 real time PCR system (Applied Biosystems), with oligonucleotide primers and probes (Roche Applied Science). The relative abundance of transcripts was normalized relative to the constitutive expression of 18 S ribosomal RNA.

Northern Blotting—Equal amounts (~5 μg) of total RNA obtained from BMMCs using TRIzol reagent were applied to separate lanes of 1.2% formaldehyde-agarose gels, electrophoresed, and transferred to Immobilon-N membranes (Millipore). The resulting blots were then probed with appropriate cDNA probes that had been labeled with [^{32}P]dCTP (PerkinElmer Life Sciences) by random priming (Takara Biomedicals). Hybridization and subsequent membrane washing were carried out as described previously (11).

Western Blotting—10^5 BMMCs were lysed in SDS-PAGE sample buffer (63 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 0.08% bromphenol blue) containing 5% 2-mercaptoethanol, and then subjected to SDS-PAGE. Proteins were subsequently blotted onto nitrocellulose membranes, followed by blocking with 5% milk powder in PBS containing 0.05% Tween 20. The membranes were incubated for 2 h with rabbit polyclonal antibody against cPLA$_2\alpha$ (Santa Cruz Biotechnology), iPLA$_2\beta$ (Cayman Chemicals), cyclooxygenase (COX)-2 (Santa Cruz Biotechnology), or mPGES-1 (Cayman Chemicals) (15, 59, 63) at 1:5000 dilution in PBS, 0.05% Tween 20. After washing with PBS, 0.05% Tween 20, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin antibody (Zymed Laboratories Inc.) at 1:5000 dilution in PBS, 0.05% Tween 20. After 1 h of incubation, the membranes were washed extensively with PBS, 0.05% Tween 20,

followed by washing with PBS without detergent. The membranes were developed with the ECL system (PerkinElmer Life Sciences) in accordance with the protocol provided by the manufacturer.

Electrospray Ionization Mass Spectrometry (ESI-MS)—Total lipids were extracted from BMMCs (10^7 cells) using the method of Bligh and Dyer (55). ESI-MS analysis was performed using a 4000Q TRAP, quadrupole-linear ion trap hybrid mass spectrometer (AB SCIEX) with an UltiMate 3000 nano/cap/microliquid chromatography system (Dionex Corp.) combined with an HTS PAL autosampler (CTC Analytics AG). Phospholipids were subjected directly to ESI-MS analysis by flow injection; typically, 3 μl (3 nmol of phosphorus equivalent) of sample was applied. The mobile phase composition was acetonitrile/methanol/water (6:7:2) (plus 0.1% ammonium formate, pH 6.8) at a flow rate of 10 μl/min. The scan range of the instrument was set at m/z 200–1000 at a scan speed of 1000 Da/s. The trap fill-time was set at 3 ms in the positive ion mode and at 5 ms in the negative ion mode. The ion spray voltage was set at 5500 V in the positive ion mode and at −4500 V in the negative ion mode. Nitrogen was used as a curtain gas (setting of 10, arbitrary units) and as a collision gas (set to "high"). Details of the procedure for ESI-MS have been described previously (56, 57).

AA Transfer Experiments—BMMCs (2×10^6 cells) were preincubated with [^{3}H]AA (0.1 μCi/ml) overnight. The ^{3}H-prelabeled BMMCs were washed with medium and then seeded onto Swiss 3T3 cells (2×10^5 cells/0.5 ml/well) in 24-well plates. After appropriate periods, BMMCs and Swiss 3T3 cells were separated by trypsinization (see above), and the radioactivity incorporated into each cell type was measured using a liquid scintillation counter (Aloka).

Passive Cutaneous Anaphylaxis—Mouse anti-DNP IgE (25 ng in 25 μl of saline) was injected intradermally into the ears of 8–12-week-old male mice. One day later, the mice were intravenously challenged with 60 ng of Ag in 200 μl of PBS containing 0.8% (w/v) Evans blue (Sigma). After 30 min, their ears were removed and dissolved, and the dye extravasation was determined colorimetrically at 630 nm, as described previously (14).

Mast Cell Knock-in—To reconstitute mast cells in mast cell-deficient mice (3, 4), 5×10^6 BMMCs were injected subcutaneously into the ears of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (The Jackson Laboratory). After 2 days, the ears were homogenized in PBS containing 10 μM indomethacin (Sigma); the supernatants were adjusted to pH 3.0 with 1 M HCl and passed through a Sep-Pak C18 cartridge (Waters), and the retained PGE_2 were eluted with 3 ml of methanol, as described previously (53). A trace amount of [^{3}H]PGE_2 (PerkinElmer Life Sciences) was added to the samples before passage through the cartridges to calibrate the recovery of the PGE_2. The sample solvents were evaporated, and then the PGE_2 was dissolved in an aliquot of buffer and assayed with EIA kit.

Statistical Analysis—Data were evaluated statistically by unpaired Student's t test at a significance level of $p < 0.05$.

RESULTS

Expression of cPLA$_2\alpha$ and iPLA$_2\beta$ in BMMCs—We examined the expression of several intracellular PLA_2 enzymes in IL-3-maintained immature BMMCs as well as those placed on Swiss 3T3 fibroblasts in the presence of SCF (designated CTMC-like cells hereafter) by RT-PCR (Fig. 1). As a positive control for the maturation of BMMCs into CTMC-like cells, we monitored the expression of mMCP-4 (*Mcpt4*) mRNA (58), whose expression was minimal in BMMCs and markedly induced after 2–4 days of coculture with fibroblasts (Fig. 1*A*). Among six members of the cPLA$_2$ family (α, β, γ, δ, ϵ, and ζ), expression of cPLA$_2\alpha$ mRNA was readily detected in BMMCs and increased modestly during the course of maturation toward CTMC-like cells (Fig. 1*A*). Expression of mRNAs for cPLA$_2\beta$ and cPLA$_2\gamma$ was barely detectable in BMMCs and was elevated (albeit still at low levels) in CTMC-like cells (Fig. 1*A*), whereas that of mRNAs for cPLA$_2\delta$, -ϵ and -ζ was below the detection limit under all conditions tested (data not shown). The prototypic iPLA$_2$ isoform, iPLA$_2\beta$, was expressed at a significant level in BMMCs and markedly elevated in CTMC-like cells (Fig. 1*A*). Quantitative RT-PCR confirmed that cPLA$_2\alpha$ and iPLA$_2\beta$ are the two major intracellular PLA_2s expressed in BMMCs (Fig. 1*B*).

The increase of cPLA$_2\alpha$ protein, as assessed by immunoblotting, paralleled that of its mRNA, reaching a plateau level by 1 day after coculture (Fig. 1*C*, *left panel*). Interestingly, immunoblotting of iPLA$_2\beta$ revealed that the full-size iPLA$_2\beta$ protein (~90 kDa), found in BMMCs on day 0, was sequentially converted to an ~60-kDa form on days 1–2 and then to an ~50-kDa form on days 3–4, with an incremental expression of the latter (Fig. 1*C*, *right panel*). Considering that iPLA$_2\beta$ is known to undergo proteolytic processing leading to its activation during cell activation or apoptosis (48, 49, 59, 60), our results may represent another example of iPLA$_2\beta$ processing during a particular cellular process, *i.e.* mast cell maturation.

It has been reported that the FcϵRI-dependent and -independent exocytotic responses are attenuated in BMMCs and RBL-2H3 cells (rat mastocytoma) by treatment with BEL, an iPLA$_2$ inhibitor (39). In addition, iPLA$_2\beta$ has been implicated in activation of the CRAC channel (41), which is essential for Ca^{2+} entry and thereby subsequent effector functions in FcϵRI-activated mast cells (61). Furthermore, contributions of iPLA$_2\beta$ to stimulus-coupled AA release, likely in concert with cPLA$_2\alpha$, have been demonstrated in vascular cells (42). To assess whether iPLA$_2\beta$ indeed participates in mast cell activation or not, we initially examined the effect of (*S*)-BEL (a preferential inhibitor of iPLA$_2\beta$) and (*R*)-BEL (a preferential inhibitor of iPLA$_2\gamma$) on β-HEX release (a marker of degranulation) by IgE/Ag-stimulated BMMCs. Both agents inhibited β-HEX release in a dose-dependent manner, with slightly better inhibition by (*S*)-BEL than by (*R*)-BEL (Fig. 1*D*).

iPLA$_2\beta$ Plays No Role in Effector Functions of Mast Cells—However, because it has become apparent that BEL is not specific for iPLA$_2\beta$ but can also broadly inhibit other iPLA$_2$ isoforms as well as some other signaling molecules and proteases (62, 63), we next attempted to clarify the role of iPLA$_2\beta$ in mast cells using *Pla2g6*$^{-/-}$ mice, in which iPLA$_2\beta$ is ablated (52). Expression of other PLA_2s expressed in BMMCs was unaffected by iPLA$_2\beta$ deficiency (supplemental Fig. S1). When IgE-sensitized BMMCs from *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice were stimulated with various concentrations of Ag, we observed no difference in β-HEX exocytosis between the genotypes at each dose (Fig. 2*A*). Likewise, IgE/Ag-dependent generation of the

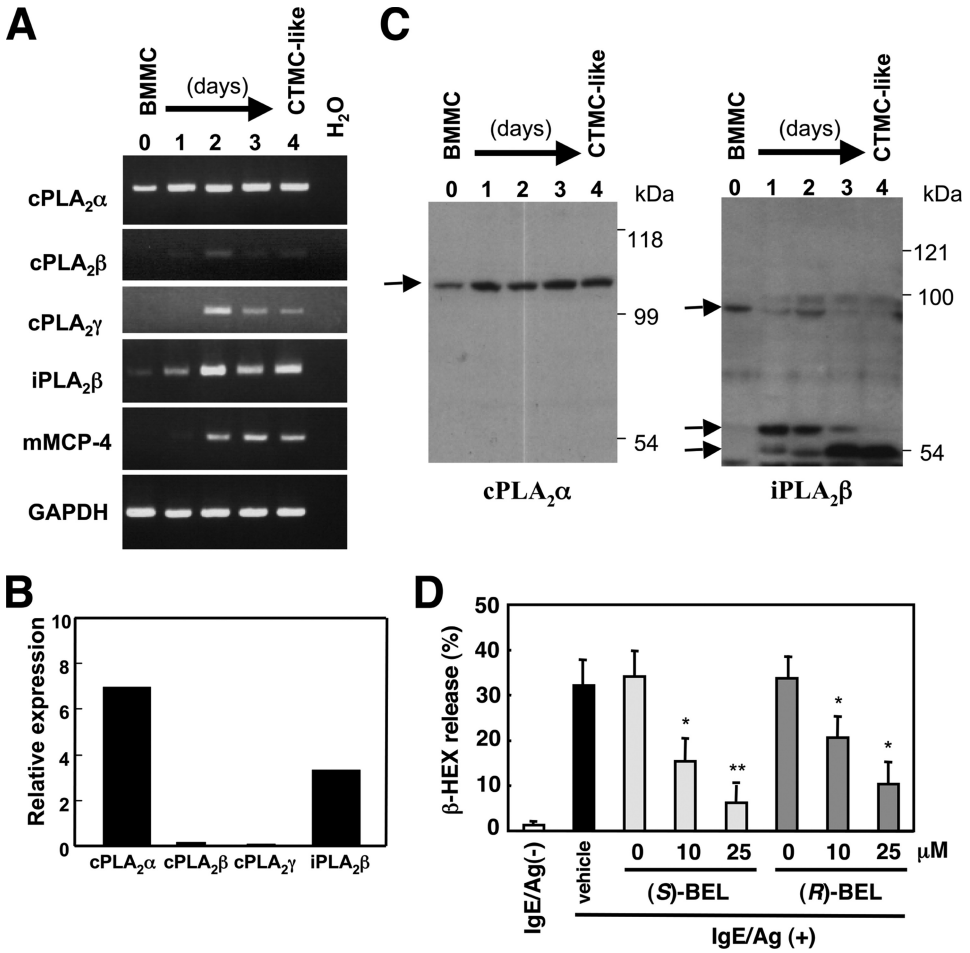

FIGURE 1. **Expression of various intracellular PLA$_2$ in mast cells.** *A*, RNAs obtained from BMMCs maintained in IL-3 and those cocultured for the indicated periods with Swiss 3T3 cells (CTMC-like cells) were subjected to RT-PCR for various PLA$_2$s (28 cycles). RT-PCR for mMCP-4, a CTMC-specific protease, was performed as a positive control for the differentiation of immature BMMCs into mature CTMCs. Equal loading of each sample was verified by RT-PCR of *Gapdh*, a housekeeping gene. RT-PCR without RNA (H$_2$O) was done as a negative control. *B*, quantitative RT-PCR of intracellular PLA$_2$s in BMMCs. *C*, lysates of replicate cells were subjected to SDS-PAGE (8% gel) followed by immunoblotting with cPLA$_2\alpha$ and iPLA$_2\beta$. *Arrows* indicate the positions of major bands for individual PLA$_2$s. *D*, effects of (*S*)- or (*R*)-BEL on β-HEX release from IgE/Ag-activated BMMCs (mean ± S.E., $n = 5$; *, $p < 0.05$ and **, $p < 0.01$).

eicosanoids, PGD_2 (Fig. 2*B*) and LTC_4 (Fig. 2*C*), did not differ appreciably between *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice. Generation of PGD_2 (data not shown) and LTC_4 (Fig. 2*D*) in response to SCF or A23187 was also approximately the same between the genotypes. Likewise, IgE/Ag-elicited secretion of IL-6 was unimpaired in *Pla2g6*$^{-/-}$ mice (Fig. 2*E*). Consistent with the normal effector functions (exocytosis, eicosanoid synthesis, and cytokine induction), the IgE/Ag-induced increase of intracellular Ca^{2+} was equivalent between *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice (Fig. 2*F*). Even after coculture with fibroblasts for 1 week, a period sufficient to induce functional maturation of mast cells (11–14), IgE/Ag-stimulated release of β-HEX (Fig. 2*G*) and generation of PGD_2 (Fig. 2*H*) and LTC_4 (data not shown) by *Pla2g6*$^{-/-}$ cells were similar to those by *Pla2g6*$^{+/+}$ cells, suggesting that the deficiency of iPLA$_2\beta$ did not affect the functional maturation of BMMCs toward CTMC-like cells. Finally, the Ag-induced PCA reaction, which depends on histamine and LTC_4 released by dermal mast cells, occurred normally in IgE-sensitized *Pla2g6*$^{-/-}$ mice (Fig. 2*I*), indicating that the differentiation and function of mast cells *in vivo* are not hampered by the absence of iPLA$_2\beta$. Thus, iPLA$_2\beta$ plays no role in mast cell activation, and the suppressive effect of BEL on degranulation by BMMCs (Fig. 1*D*) appears to result from its off-target effects, rather than from inhibition of iPLA$_2\beta$.

Role of cPLA$_2\alpha$, but Not iPLA$_2\beta$, in FcϵRI-activated AA Mobilization from Mast Cell Membranes—To assess the lipid dynamics during IgE/Ag activation of BMMCs, lipids extracted from BMMCs with or without IgE/Ag stimulation for 2 min (a time point when the rate of AA release was maximal (64)) were subjected to ESI-MS analyses. Quantification of average scores ($n = 4$) for individual PC molecular peaks revealed that, in wild-type BMMCs, all phosphatidylcholine (PC) species containing *sn*-2 AA (20:4) or eicosapentaenoic acid (EPA; 20:5) were significantly decreased (Fig. 3*A*), with a concomitant increase in LPC molecular species (Fig. 3*C*), following IgE/Ag treatment. In contrast, PC molecular species with *sn*-2 palmitic acid (16:0), stearic acid (18:0), oleic acid (OA; C18:1), linoleic acid (LA; 18:2), and docosahexaenoic acid (22:6) were unchanged irrespective of the stimulation (Fig. 3, *A* and *B*). It is known that cPLA$_2\alpha$ shows selectivity for *sn*-2 AA- and EPA-bearing phospholipids (65), and in fact, the stimulus-dependent selective reductions of AA- and EPA-containing PC species as well as increases in LPC species were entirely absent in BMMCs from *Pla2g4a*$^{-/-}$ mice (Fig. 3, *A–C*). Thus, these lipidomic studies

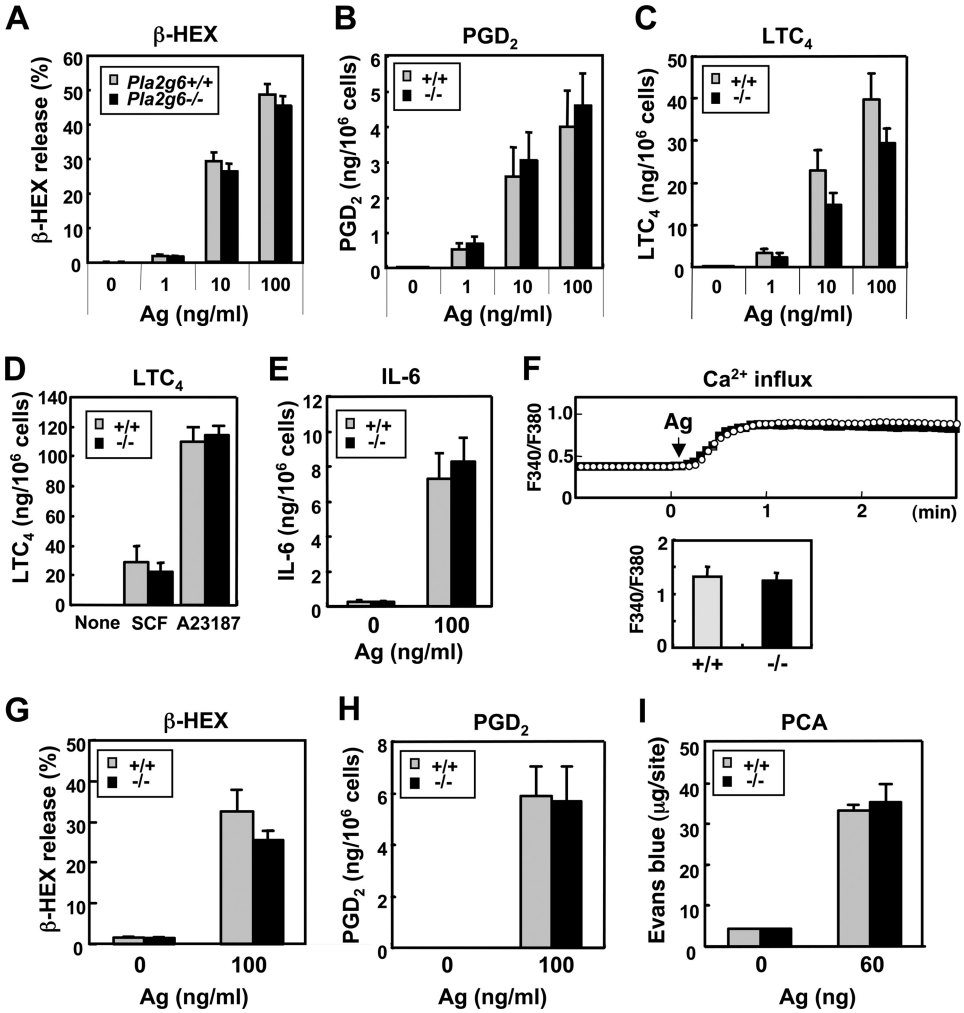

FIGURE 2. **iPLA$_2\beta$ plays no role in the effector functions of mast cells.** *A–C*, IgE-sensitized BMMCs from *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice were challenged with the indicated concentrations of Ag for 10 min, and the releases of β-HEX (*A*), PGD_2 (*B*), and LTC_4 (*C*) into the supernatants were evaluated (mean ± S.D., $n = 5–6$). *D*, LTC_4 generation by *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ BMMCs treated for 10 min with 100 ng/ml SCF or 1 μM A23187 (mean ± S.D., $n = 3$). *E*, IgE/Ag-stimulated BMMCs from *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice were cultured for 10 h in the presence of SCF and IL-1β (which amplify cytokine expression in BMMCs (63)) to assess IL-6 secretion (mean ± S.D., $n = 3$). *F*, IgE/Ag-triggered Ca^{2+} influx into BMMCs. A representative monitoring of intracellular Ca^{2+} levels in *Pla2g6*$^{+/+}$ (*white circles*) and *Pla2g6*$^{-/-}$ (*solid circles*) BMMCs after Ag challenge (*upper panel*) and an average value at 3 min (mean ± S.E.; $n = 3$) (*lower panel*) are shown. *G* and *H*, IgE-sensitized *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ CTMC-like cells, which had been cocultured for 1 week with Swiss 3T3 fibroblasts, were treated with or without Ag for 10 min, and the releases of β-HEX (*G*) and PGD_2 (*H*) into the supernatants were evaluated (mean ± S.D., $n = 3$). *I*, IgE-sensitized *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice were challenged with or without Ag to assess PCA reaction (mean ± S.D., $n = 5$).

confirmed that most of the FcεRI-dependent AA release by mast cells is mediated by cPLA$_2\alpha$ (35, 36).

Although it has been reported that prior activation of iPLA$_2\beta$ and thereby gating of the CRAC channel may be important for full activation of cPLA$_2\alpha$ in vascular smooth muscle cells (41, 42), it is unlikely that the iPLA$_2\beta$-cPLA$_2\alpha$ axis is operative in BMMCs because, in agreement with unaltered Ca^{2+} response and effector functions (Fig. 2), IgE/Ag-evoked decreases in AA- or EPA-containing PC species and reciprocal increases in LPC species occurred almost normally in *Pla2g6*$^{-/-}$ BMMCs (Fig. 3, *D–F*). An exception was the propensity for less reduction of some PC species with AA (alkyl 16:0–20:4 and alkyl 18:1–20:4) in IgE/Ag-activated *Pla2g6*$^{-/-}$ BMMCs in comparison with replicate wild-type BMMCs (Fig. 3*D*). This subtle change might be related to the modestly decreasing trend of LTC_4 synthesis in *Pla2g6*$^{-/-}$ BMMCs (Fig. 2*C*), although we did not investigate this issue further because the difference in LTC_4 synthesis between the genotypes did not reach statistical significance.

Role of cPLA$_2\alpha$, but Not iPLA$_2\beta$, in Phospholipid Remodeling during Mast Cell Maturation—We next examined whether the elevated expression of cPLA$_2\alpha$ or iPLA$_2\beta$ in BMMCs during *in vitro* maturation would be associated with some compositional changes in membrane phospholipids. To this end, total lipids were extracted from BMMCs (*i.e.* maintained in the presence of IL-3 without coculture) and CTMC-like differentiating cells (*i.e.* after coculture with fibroblasts plus SCF for 4 days) and then subjected to ESI-MS analyses. Representative ESI-MS profiles of phospholipids (PC, phosphatidylethanolamine (PE), and phosphatidylserine (PS)) in mast cells from wild-type mice are shown in Fig. 4, which indicated that several if not all PC and PE molecular species containing *sn*-2 AA were decreased, whereas those with *sn*-2 OA or LA were conversely increased, in CTMC-like cells in comparison with IL-3-maintained BMMCs. In contrast, there were no alterations in PS molecular species between BMMCs and CTMC-like cells (Fig. 4).

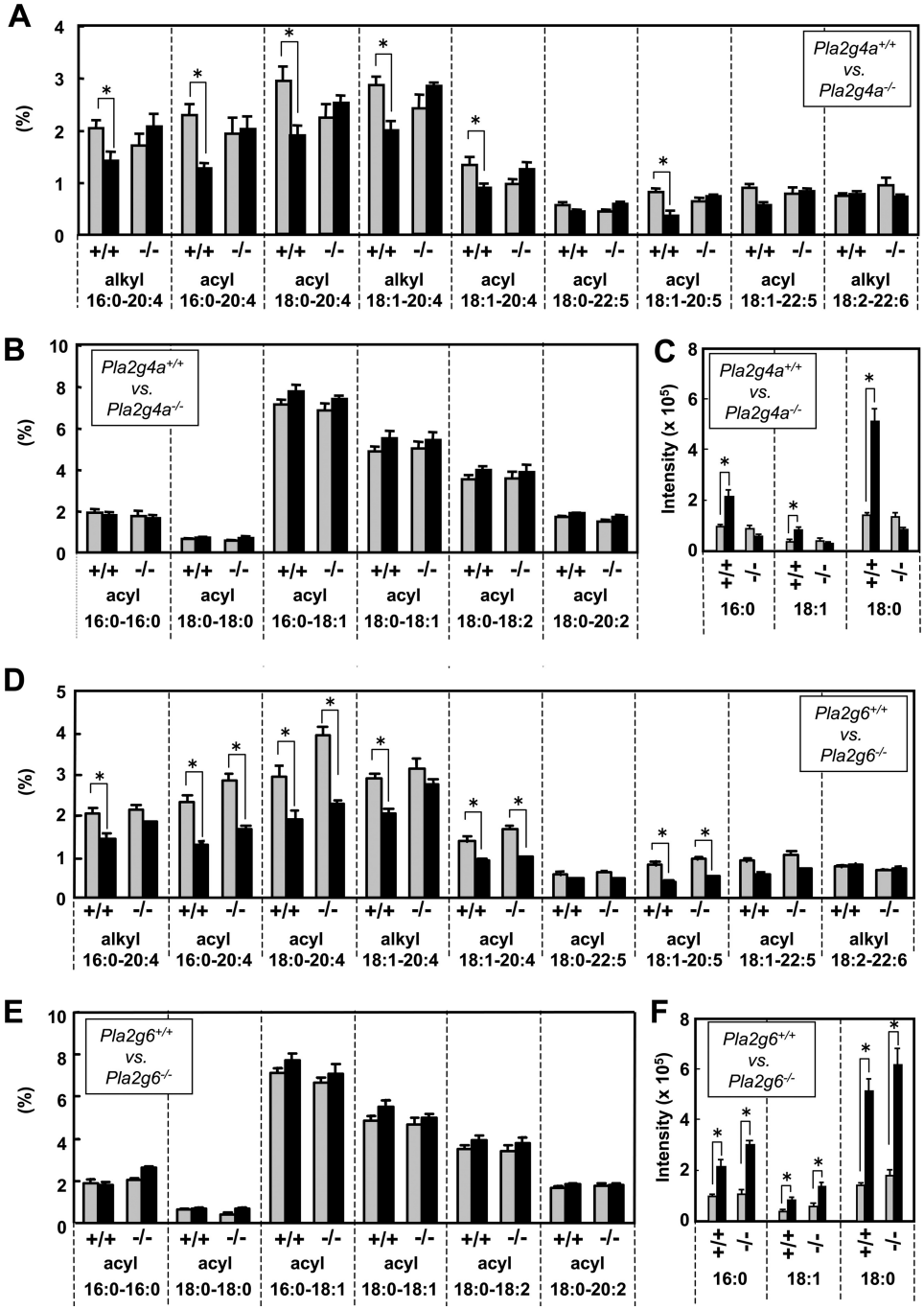

FIGURE 3. **ESI-MS analysis of PC molecular species in BMMCs with or without IgE/Ag stimulation.** BMMCs from *Pla2g4a*$^{+/+}$ and *Pla2g4a*$^{-/-}$ mice (*A–C*) or from *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice (*D–F*) were sensitized with IgE and then treated with (*filled bars*) or without (*gray bars*) 100 ng/ml Ag for 2 min. Lipids extracted from these cells were subjected to ESI-MS on a positive ion mode. PC species bearing *sn*-2 fatty acids with a high degree (4) of unsaturation (*A* and *D*), those bearing *sn*-2 fatty acids with no or a low degree (2) of unsaturation (*B* and *E*), and LPC species (*C* and *F*) were quantified. Values indicate the percentages of individual PC species relative to total PC mass (*A, B, D,* and *E*) or signal intensities of individual LPC species on ESI-MS (*C* and *F*) (mean ± S.D., $n = 3–4$; *, $p < 0.05$).

The results shown in Fig. 4 were further validated by quantification of average scores ($n = 4$) for the proportion of individual PC molecular species (Fig. 5). In CTMC-like cells, several if not all PC molecular species with *sn*-2 AA and EPA, namely PC with diacyl 16:0–20:4, alkyl 16:0–20:4, alkyl 18:1–20:4, and diacyl 18:1–20:5, were selectively and significantly decreased relative to those in IL-3-maintained BMMCs (Fig. 5*A*). In contrast, CTMC-like cells contained more PC molecular species with diacyl 18:0–18:1 and 18:0–18:2 than did BMMCs (Fig. 5*B*). Thus, maturation of BMMCs toward CTMC-like cells was accompanied by decreased unsaturation of *sn*-2 fatty acids in phospholipids. Notably, the decrease of AA-containing PC species was not seen in *Pla2g4a*$^{-/-}$ CTMC-like cells (Fig. 5*A*), suggesting that cPLA$_2\alpha$ is mostly responsible for the liberation of AA from phospholipids during mast cell maturation. However, the

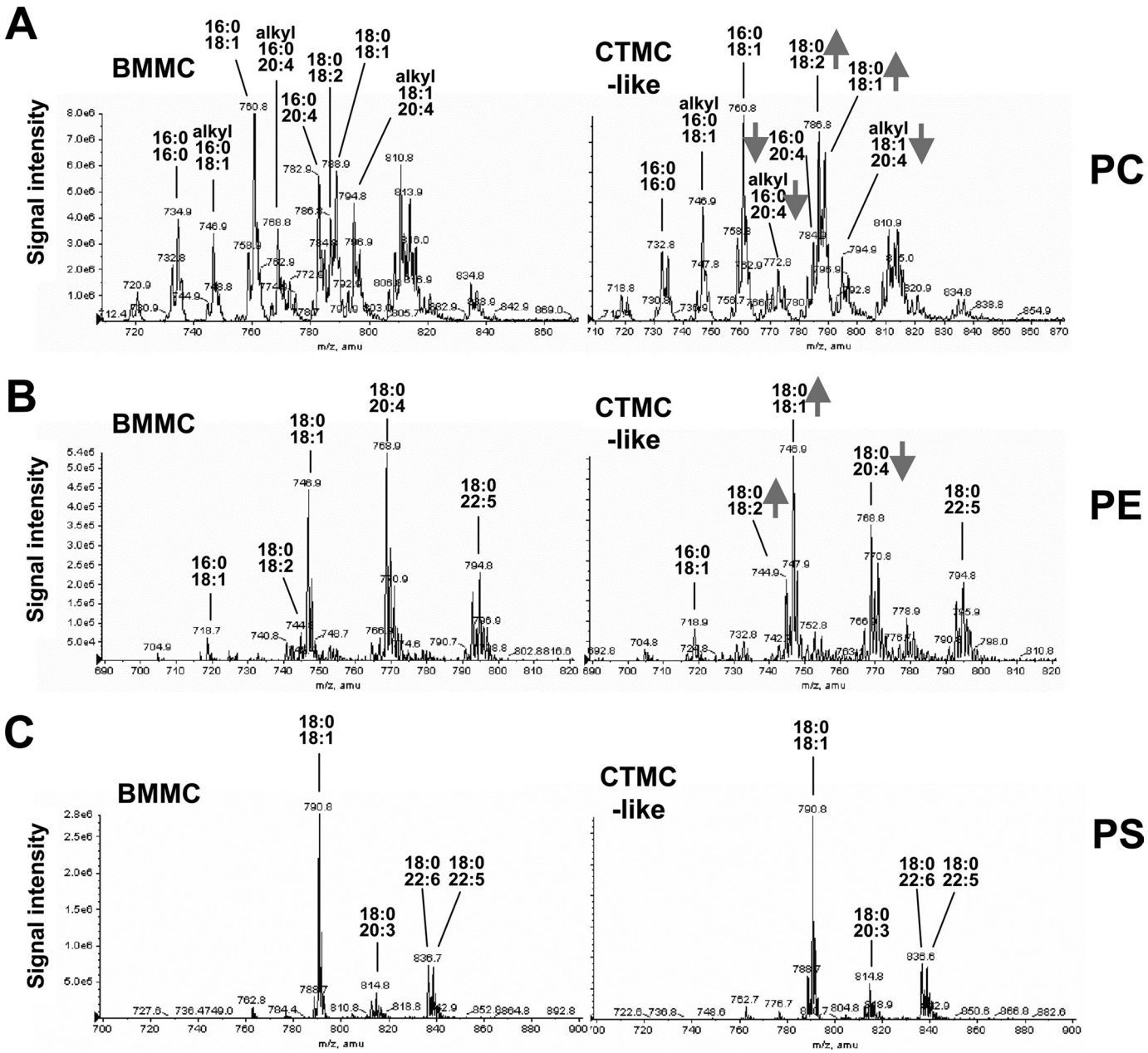

FIGURE 4. **ESI-MS profiles of phospholipids in BMMCs and CTMC-like cells from C57BL/6 mice.** IL-3-maintained BMMCs (*left panel*) and CTMC-like differentiated cells after coculture for 4 days with Swiss 3T3 cells (*right panel*) were subjected to ESI-MS for PC, PE, and PS. Fatty acid compositions of major peaks are indicated. Peaks reduced or increased in CTMC-like cells relative to BMMCs are shown by *arrows*.

decrease of PC with EPA and the increase of PC with OA and LA were not influenced by the deficiency of cPLA$_2\alpha$ (Fig. 5, *A* and *B*), suggesting that these processes are regulated by other PLA$_2$(s) and/or acyltransferase(s). Importantly, these changes in PC molecular species were not impaired at all in *Pla2g6*$^{-/-}$ cells (Fig. 5, *C* and *D*). Thus, although iPLA$_2\beta$ has been believed to be a regulator of phospholipid membrane remodeling (40), it does not contribute significantly to this process in mast cells.

Mast Cell cPLA$_2\alpha$ Is Coupled with Fibroblastic mPGES-1-dependent PGE$_2$ Production—Having established that AA-containing phospholipids are selectively decreased by cPLA$_2\alpha$ during coculture of BMMCs with fibroblasts, we next examined whether the free AA thus released would be metabolized to PGD$_2$, a main mast cell-produced prostanoid. However, the level of PGD$_2$ was low in the supernatants of BMMC-fibroblast cocultures (Fig. 6*A*, *left panel*). Unexpectedly, the level of PGE$_2$, which was produced to some extent by Swiss 3T3 cells alone and not by BMMCs, was markedly increased in the coculture supernatants (Fig. 6*A*, *right panel*). Addition of the coculture supernatants to fresh Swiss 3T3 cells did not increase the PGE$_2$ level further (data not shown), suggesting that the increased PGE$_2$ synthesis requires direct contact between BMMCs and fibroblasts and that it is not mediated by cytokines or other soluble factors secreted from BMMCs.

Expression of a panel of enzymes involved in PGE$_2$ biosynthesis in BMMCs and Swiss 3T3 cells before and after cocul-

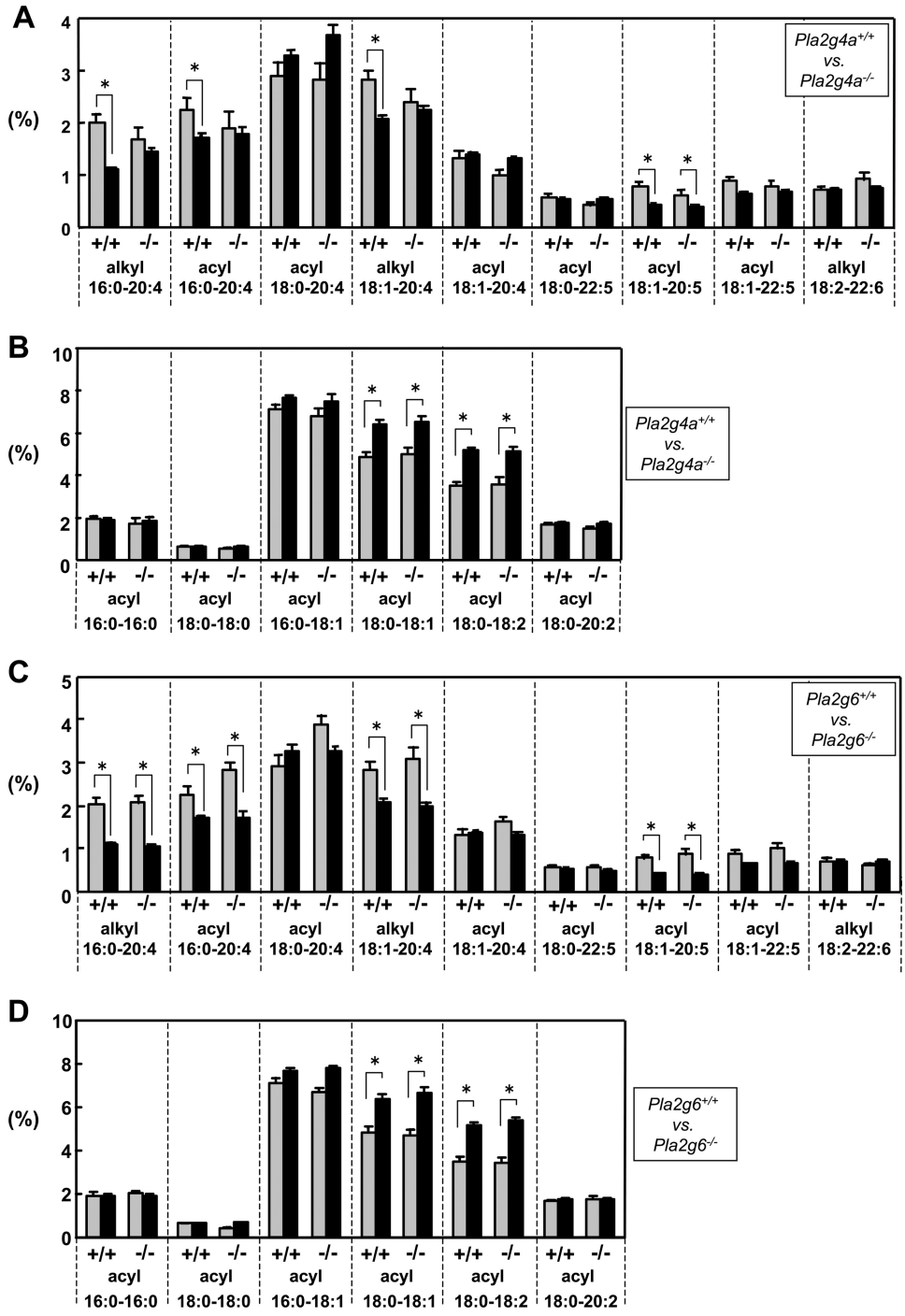

FIGURE 5. **ESI-MS analysis of PC molecular species in BMMCs and CTMC-like cells.** IL-3-maintained BMMCs (*gray bars*) and CTMC-like differentiated cells after coculture for 4 days with Swiss 3T3 cells in the presence of SCF (*filled bars*) from *Pla2g4a*$^{+/+}$ and *Pla2g4a*$^{-/-}$ mice (*A* and *B*) or from *Pla2g6*$^{+/+}$ and *Pla2g6*$^{-/-}$ mice (*C* and *D*) were subjected to ESI-MS analyses for PC molecular species on a positive ion mode. PC species bearing *sn*-2 fatty acids with a high degree (4) of unsaturation (*A* and *C*) and those bearing *sn*-2 fatty acids with no or a low degree (2) of unsaturation (*B* and *D*) were quantified. Values indicate the percentages of individual PC species relative to total PC mass (mean ± S.D., $n = 3–4$; *, $p < 0.05$).

ture, as assessed by immunoblotting, is shown in Fig. 6*B*. In BMMCs, cPLA$_2\alpha$ (also see Fig. 1*B*) and COX-2 were increased significantly after coculture with fibroblasts, whereas mPGES-1, a terminal enzyme crucial for isomerization of COX-2-produced PGH$_2$ to PGE$_2$, was barely detectable regardless of culture conditions (Fig. 6*B*, *left panel*). All three of these enzymes were constitutively expressed in Swiss 3T3 cells, in which only cPLA$_2\alpha$ exhibited an obvious increase after coculture (Fig. 6*B*, *right panel*). The almost complete absence of mPGES-1 expression in BMMCs suggested that Swiss 3T3 cells, but not BMMCs, were the main source of PGE$_2$ during coculture.

To confirm this finding, we utilized BMMCs obtained from mPGES-1-deficient (*Ptges*$^{-/-}$) mice. When BMMCs from *Ptges*$^{+/+}$ and *Ptges*$^{-/-}$ mice were each cocultured with Swiss 3T3 cells, the levels of PGE$_2$ released into the coculture super-

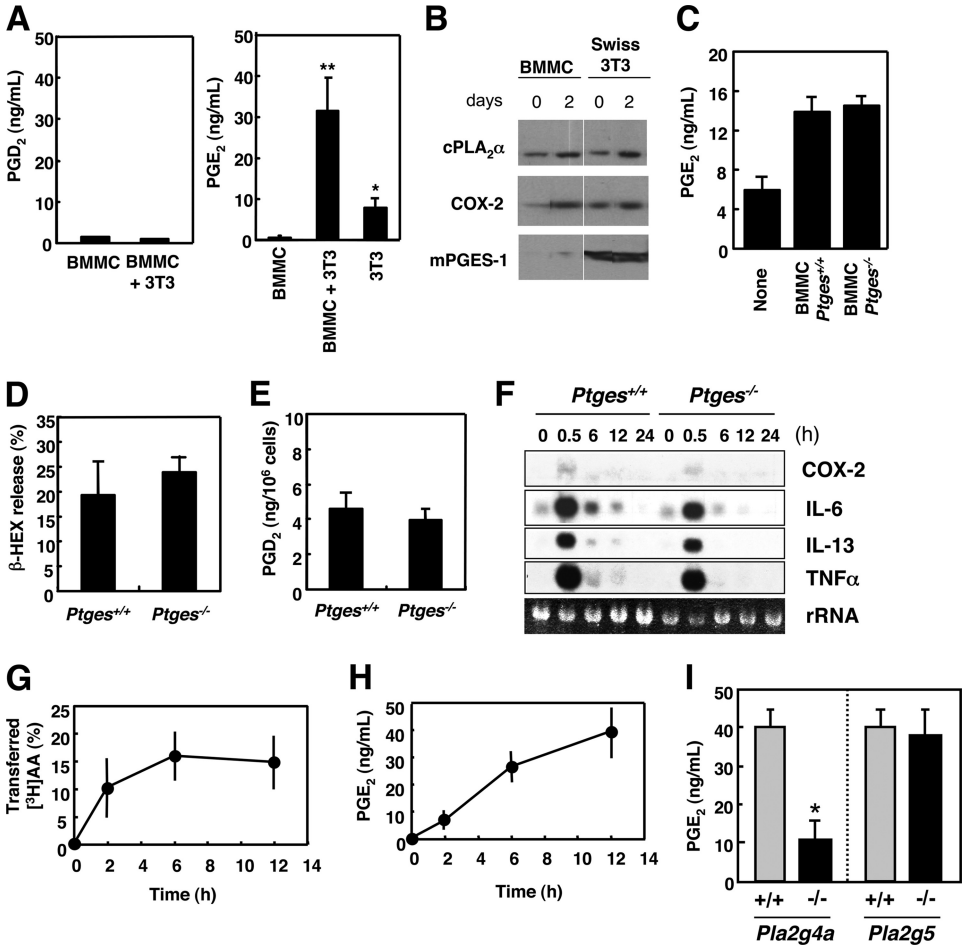

FIGURE 6. **cPLA$_2\alpha$ in mast cell is coupled with mPGES-1 in fibroblasts to provide anti-allergic PGE$_2$.** *A*, BMMCs alone, BMMCs plus Swiss 3T3 cells, and Swiss 3T3 alone were cultured for 2 days, and the resultant supernatants were taken for enzyme immunoassay for PGD_2 (*left panel*) or PGE_2 (*right panel*). Means ± S.D., $n = 6$; *, $p < 0.01$ *versus* BMMCs, or Swiss 3T3 cells alone and **, $p < 0.01$ *versus* BMMCs alone. *B*, expression of PGE_2 biosynthetic enzymes in BMMCs and Swiss 3T3 cells before and 2 days after coculture, as assessed by immunoblotting. *C*, PGE_2 production by Swiss 3T3 cells alone and those cocultured with *Ptges*$^{+/+}$ or *Ptges*$^{-/-}$ BMMCs (mean ± S.D., $n = 2$). *D* and *E*, BMMCs from *Ptges*$^{+/+}$ and *Ptges*$^{-/-}$ mice were sensitized with IgE and stimulated with Ag for 15 min to assess β-HEX exocytosis (*D*) and PGD_2 generation (*E*) (mean ± S.D., n = 8-~12). *F*, total RNAs extracted from *Ptges*$^{+/+}$ and *Ptges*$^{-/-}$ BMMCs after stimulation with Ag for the indicated periods were subjected to Northern blotting for COX-2 and various cytokines (IL-6, IL-13, and TNFα). *rRNA*, 28 S ribosomal RNA visualized in an agarose gel with ethidium bromide. A representative result of three independent experiments is shown. *G*, [^{3}H]AA-prelabeled BMMCs were cocultured with Swiss 3T3 cells, and percentage transfer of [^{3}H]AA to Swiss 3T3 cells relative to total incorporation of [^{3}H]AA into BMMCs was monitored over time (mean ± S.D., $n = 4$). *H*, time course of PGE_2 synthesis by BMMC-Swiss 3T3 coculture (mean ± S.D., $n = 4$). *I*, BMMCs obtained from *Pla2g4a*$^{-/-}$ ($n = 8$) and *Pla2g5*$^{-/-}$ ($n = 4$) mice and their littermate wild-type controls were cocultured with Swiss 3T3 cells for 12 h to assess the coculture-dependent increase in PGE_2 release (mean ± S.D., *, $p < 0.05$ *versus Pla2g4a*$^{+/+}$ control).

natants were comparable (Fig. 6*C*), implying that the augmented PGE_2 during coculture originated from Swiss 3T3 cells and not from BMMCs. Furthermore, although previous studies have shown that PGE_2 promotes the differentiation of BMMCs from bone marrow cells (66) and modulates the effector functions of BMMCs (67–69), IL-3- or SCF-dependent growth (data not shown) and FcεRI-dependent exocytosis of β-HEX (Fig. 6*D*), generation of PGD_2 (Fig. 6*E*), and transcriptional induction of COX-2 and the cytokines IL-6, IL-13, and TNFα (all of which attained a maximal expression at 0.5 h after stimulation, as assessed by Northern blotting) (Fig. 6*F*) in *Ptges*$^{-/-}$ BMMCs were similar to those in replicate *Ptges*$^{+/+}$ BMMCs, suggesting that the genetic deletion of mPGES-1 in BMMCs does not affect their proliferation and effector functions.

The results described above led us to hypothesize that the AA released by cPLA$_2\alpha$ in BMMCs is transferred to Swiss 3T3 cells and is then metabolized to PGE_2. To address whether AA would indeed be transferred from mast cells to fibroblasts, BMMCs were prelabeled with [^{3}H]AA, washed, and then cocultured with Swiss 3T3 cells. A short (hours) rather than long (days) time course was chosen for this experiment to minimize any possibility of metabolic degradation of [^{3}H]AA. As shown in Fig. 6*G*, [^{3}H]AA was readily transferred from BMMCs to cocultured Swiss 3T3 cells within hours. This [^{3}H]AA transfer was followed by a concomitant increase of PGE_2 synthesis in BMMC-3T3 coculture (Fig. 6*H*).

To acquire clear evidence for the contribution of mast cell-associated cPLA$_2\alpha$ to fibroblastic PGE_2 synthesis, BMMCs obtained from *Pla2g4a*$^{-/-}$ and *Pla2g4a*$^{+/+}$ mice were cocultured with Swiss 3T3 cells. We also used BMMCs obtained from group V sPLA$_2$-null (*Pla2g5*$^{-/-}$) mice, because it has been reported that group V sPLA$_2$, which is expressed in BMMCs, is able to promote transcellular eicosanoid synthesis by adjacent cells (38, 70, 71). As shown in Fig. 6*I*, the augmented PGE_2 production in coculture was partially reduced when Swiss 3T3 cells were incubated with *Pla2g4a*$^{-/-}$ BMMCs relative to that

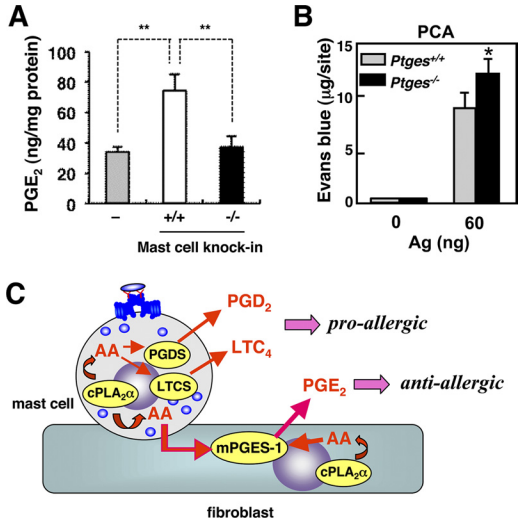

FIGURE 7. **Mast cell cPLA$_2\alpha$-dependent biosynthesis of anti-anaphylactic PGE$_2$ *in vivo*.** *A*, *Pla2g4a*$^{+/+}$ or *Pla2g4a*$^{-/-}$ BMMCs were subcutaneously transferred into the ears of mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. After 2 days of reconstitution, PGE$_2$ levels in the ears of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (−), those transferred with *Pla2g4a*$^{+/+}$ BMMCs (+/+), and those transferred with *Pla2g4a*$^{-/-}$ BMMCs (−/−) were quantified (mean ± S.E., $n = 12$, **, $p < 0.01$). *B*, IgE-sensitized *Ptges*$^{+/+}$ and *Ptges*$^{-/-}$ mice were challenged with or without Ag to assess PCA reaction (mean ± S.E., $n = 3$; *, $p < 0.05$ *versus* replicate *Ptges*$^{+/+}$ mice). *C*, schematic diagram of the dual role of cPLA$_2\alpha$ in mast cells for the production of pro-allergic and anti-allergic lipid mediators. In mast cells, cPLA$_2\alpha$ supplies AA (via COX-1 and -2, which are omitted in the figure) to hematopoietic PGD$_2$ synthase (*PGDS*) and LTC$_4$ synthase (*LTCS*) for the biosynthesis of pro-allergic PGD$_2$ and LTC$_4$, respectively (64). In adjacent fibroblasts, AA is supplied by cPLA$_2\alpha$ intrinsically expressed in fibroblasts (cell autonomous pathway) and by cPLA$_2\alpha$ in mast cells (transcellular pathway) to mPGES-1 for the biosynthesis of anti-allergic PGE$_2$.

in coculture with control BMMCs, suggesting that a significant proportion of fibroblastic PGE$_2$ is derived from AA released by cPLA$_2\alpha$ in mast cells and that the remaining PGE$_2$ in coculture with *Pla2g4a*$^{-/-}$ BMMC may be produced by activation of cPLA$_2\alpha$ in fibroblasts. In contrast, the PGE$_2$-synthetic responses in Swiss 3T3 cells cocultured with *Pla2g5*$^{-/-}$ BMMCs and those cocultured with control BMMCs were comparable (Fig. 6*I*), ruling out the contribution of group V sPLA$_2$ to mast cell-driven fibroblastic (transcellular) PGE$_2$ synthesis in this setting.

To address whether the mast cell cPLA$_2\alpha$-dependent transcellular PGE$_2$ synthesis could occur *in vivo*, we carried out the adoptive transfer of *Pla2g4a*$^{+/+}$ or *Pla2g4a*$^{-/-}$ BMMCs into mast cell-deficient mice (mast cell knock-in) (3, 4). Thus, BMMCs from either genotype were transferred subcutaneously into the ears of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, and after 2 days of this mast cell reconstitution, PGE$_2$ levels in the ear homogenates were quantified. As shown in Fig. 7*A*, a substantial level of PGE$_2$ was already present in the ears of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, indicating that this PGE$_2$ pool is independent of mast cells. Strikingly, the PGE$_2$ level was nearly doubled in the ears reconstituted with wild-type BMMCs, whereas *Pla2g4a*$^{-/-}$ BMMCs were unable to increase it (Fig. 7*A*). Proper reconstitution of BMMCs from both genotypes in *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice was verified by equal histamine levels (data not shown). These results provide evidence that mast cells have the ability to mobilize microenvironmental PGE$_2$ synthesis *in vivo* and that this process depends on cPLA$_2\alpha$ in mast cells.

To assess the role of mPGES-1-derived PGE$_2$ in the mast cell-dependent allergic response *in vivo*, we compared IgE/Ag-induced PCA reaction in *Ptges*$^{+/+}$ and *Ptges*$^{-/-}$ mice. As shown in Fig. 7*B*, extravasation of Evans blue dye was modestly but significantly enhanced in Ag-challenged *Ptges*$^{-/-}$ mice in comparison with replicate *Ptges*$^{+/+}$ mice. Thus, although the mast cell-produced eicosanoids PGD$_2$ and LTC$_4$ exert pro-allergic actions (23, 27, 28), mPGES-1-driven PGE$_2$ produced by stromal fibroblasts, likely through fibroblast-associated intrinsic cPLA$_2\alpha$ and through mast cell-resident extrinsic cPLA$_2\alpha$, has a counter-regulatory role in the anaphylactic response (Fig. 7*C*).

Participation of iPLA$_2\beta$ in PGE$_2$ Production by Dendritic Cells and Macrophages—Finally, to ask whether the apparent absence of iPLA$_2\beta$ requirement is specific for mast cells or could be relevant to other cell types, we investigated the PGE$_2$ biosynthetic response by other bone marrow-derived cells, *i.e.* dendritic cells and macrophages, from *Pla2g6*$^{-/-}$ mice as well as from *Pla2g4a*$^{-/-}$ mice for comparison. To this end, GM-CSF-induced BMDCs or macrophage colony-stimulating factor-induced BMMΦs from *Pla2g6*$^{-/-}$ mice, *Pla2g4a*$^{-/-}$ mice, or their littermate controls were stimulated with LPS (a ligand for TLR4) or zymosan (a ligand of TLR2 and dectin-1). As shown in supplemental Fig. S2*A*, LPS-stimulated PGE$_2$ production by *Pla2g6*$^{-/-}$ BMDCs or BMMΦs was partially reduced relative to that by replicate control cells, whereas zymosan-stimulated PGE$_2$ production was unaffected by iPLA$_2\beta$ deficiency. In comparison, LPS-stimulated PGE$_2$ release was more profoundly, if not solely, dampened in *Pla2g4a*$^{-/-}$ BMDCs or BMMΦs, whereas almost no production of PGE$_2$ was evident in these cells in response to zymosan (supplemental Fig. S2*B*). These results suggest that iPLA$_2\beta$ is partially responsible for LPS-, not zymosan-, stimulated PGE$_2$ generation in dendritic cells and macrophages. Thus, the participation of iPLA$_2\beta$ in eicosanoid synthesis may depend on cell types or stimuli (see "Discussion").

DISCUSSION

This study has revealed several new lipid-related processes that occur in mast cells during activation after FcεRI cross-linking and during maturation after mast cell-fibroblast interaction as follows. (i) BMMCs express the two intracellular PLA$_2$s, cPLA$_2\alpha$ and iPLA$_2\beta$, and interaction of BMMCs with fibroblasts results in increased expression of both enzymes (Fig. 1). (ii) Following IgE/Ag stimulation of BMMCs, cPLA$_2\alpha$ regulates most of the AA-selective release, whereas iPLA$_2\beta$ plays minimal roles (Figs. 2 and 3). (iii) Maturation of BMMCs toward CTMC-like cells is accompanied by phospholipid remodeling, with reduction of AA-containing phospholipid species and a reciprocal increase of OA- and LA-containing species (Figs. 4 and 5). (iv) A significant proportion (if not all) of these maturation-related changes in membrane phospholipids is regulated by cPLA$_2\alpha$, whereas iPLA$_2\beta$ is again dispensable for this process (Fig. 5). (v) During maturation, the AA released by cPLA$_2\alpha$ in CTMC-like differentiating cells is supplied to adjacent fibroblasts to be metabolized to PGE$_2$ via mPGES-1 (Fig. 6). (vi) Finally, the mPGES-1-derived PGE$_2$ can counteract the local anaphylactic reaction (Fig. 7). Although the molecular

mechanism underlying the anti-anaphylactic action of PGE_2 *in vivo* has not yet been fully understood, it appears to be mediated by the PGE receptor EP3, because its absence also results in exacerbated allergic reactions in mice (31). Reportedly, the deficiency of mPGES-1 also leads to augmented asthmatic airway inflammation, which could be explained by profound alteration in vascular remodeling (32). Thus, the augmented PCA reaction in *Ptges*$^{-/-}$ mice observed in our study might also rely on a similar vascular mechanism. Importantly, our present results suggest that not only do mast cells produce pro-allergic PGD_2 and LTC_4 in response to particular stimuli, such as FcεRI cross-linking, cytokines, and neurotransmitters, but they also have the capacity to modulate the microenvironmental synthesis of anti-allergic PGE_2, thereby controlling physiological balance through cell-autonomous and -extrinsic mechanisms involving lipid mediators.

It has been established that cPLA$_2\alpha$ plays a central role in eicosanoid synthesis by activated mast cells, because BMMCs from *Pla2g4a*$^{-/-}$ mice produce minimal amounts of PGD_2 and cysteinyl LTs following IgE-dependent and -independent stimuli (35, 36). In this study, we found that cPLA$_2\alpha$ in mast cells can also participate in PGE_2 synthesis by adjacent fibroblasts through a cell-to-cell AA transfer mechanism on the basis of the following findings. First, as pointed out above, AA-containing PC and PE were selectively reduced in CTMC-like differentiated cells after coculture with fibroblasts (Figs. 4 and 5). This change was largely abrogated in *Pla2g4a*$^{-/-}$ CTMC-like cells, in agreement with the view that AA selectivity is one of the most notable characteristics of cPLA$_2\alpha$ among the PLA_2 enzymes reported so far (33, 65). The PS composition of BMMCs before and after coculture remained unaltered, probably because AA-containing molecular species in PS are present at very low levels in these cells (Fig. 4). Second, [^{3}H]AA pre-incorporated into the membranes of BMMCs was readily transferred to fibroblasts during coculture, accompanied by generation of PGE_2 (Fig. 6, *G* and *H*). Third, a study using BMMCs from *Ptges*$^{-/-}$ mice clearly indicated that the augmented PGE_2 detected in the coculture supernatants was entirely derived from fibroblasts, and not from mast cells (Fig. 6*C*). Finally, and most importantly, *Pla2g4a*$^{-/-}$ BMMCs did not fully support the augmented PGE_2 generation upon coculture with fibroblasts (Fig. 6*I*) or upon adoptive transfer into the skin of mast cell-deficient mice (Fig. 7*A*). Although mast cells express group V sPLA$_2$, which reportedly has the potential to act on neighboring cells through the transcellular route after secretion (38, 70, 71), our study using *Pla2g5*$^{-/-}$ BMMCs argues against the contribution of this sPLA$_2$ isoform to coculture augmented fibroblastic PGE_2 production. These observations suggest that AA is released mainly by cPLA$_2\alpha$ from phospholipids in mast cells, transferred to proximal fibroblasts through the juxtacrine route, and then sequentially metabolized to PGE_2 by fibroblastic mPGES-1. As COX-2 was present in both BMMCs and fibroblasts after coculture (Fig. 6*B*), it is also possible that the cPLA$_2\alpha$-released AA is metabolized by COX-2 in BMMCs to PGH_2, which, despite its instability, is then transferred and converted to PGE_2 by mPGES-1 in fibroblasts.

The transfer of the intermediate PGH_2 or LTA_4 from endothelial cells to blood cells, or vice versa, has been demonstrated in cultured cells *in vitro* and in organ cultures *ex vivo* (72), and the physiological relevance of this system has recently gained a rationale by sophisticated approaches using adoptive transfer of bone marrow cells from mice lacking enzymes in the 5-lipoxygenase pathway (73, 74). However, even though these bone marrow chimera studies have delineated the transcellular eicosanoid synthesis between hematopoietic cells and parenchyma cells during inflammation, it remains unclear as to which types of hematopoietic cells and which subtypes of PLA_2 are actually involved in this process. To our knowledge, this study is the first to demonstrate that cPLA$_2\alpha$ in mast cells is capable of regulating prostanoid synthesis by neighboring fibroblasts. Thus, although mast cells can modulate the proliferation and functions of adjacent fibroblasts by several ways (15–20), the transcellular PGE_2 synthesis observed in this study represents a novel aspect of mast cell-fibroblast communication.

Despite notable decreases in the proportion of AA-bearing phospholipid species in CTMC-like differentiating cells relative to IL-3-maintained immature BMMCs, there was no accumulation of lysophospholipids in CTMC-like cells (data not shown), suggesting that lysophospholipids are rapidly cleared from the cells by either reacylation or hydrolytic degradation. The occurrence of lysophospholipid reacylation was supported by the fact that PC containing OA and LA, nutrient fatty acids that can be supplied abundantly from the culture medium, are reciprocally increased in CTMC-like cells. Because cPLA$_2\gamma$, an enzyme that is induced substantially (albeit at a low level) in CTMC-like cells (Fig. 1*A*), possesses strong lysophospholipase and transacylase activities (75), it might be at least partly responsible for the removal of lysophospholipids in these cells. Although the physiological significance of the enrichment of OA- and LA-bearing phospholipids in CTMC-like cells is unclear, it might be related to the increase in granule membranes in mature mast cells. Nevertheless, despite the decreased proportion of AA-containing phospholipids in CTMC-like cells, they are able to produce PGD_2 abundantly after IgE/Ag stimulation (Fig. 2*H*), implying that the residual AA pool in CTMC-like cells is sufficient for their synthesis of lipid mediators. Unlike FcεRI-dependent activation in which virtually all AA-containing PC species are promptly decreased (Fig. 3), some AA-containing PC species such as diacyl-PC with 18:0–20:4 or 18:1–20:4 did not decrease or rather showed a tendency to increase modestly in CTMC-like cells compared with BMMCs, suggesting that these phospholipid species are compartmentalized in certain membrane domains to which cPLA$_2\alpha$ is hardly accessible or that they are produced from some other phospholipid species by a process involving AA reacylation.

Although iPLA$_2\beta$ was originally proposed to be the main enzyme responsible for phospholipid remodeling, and thereby for the maintenance of cellular homeostasis (40), none of our present results support the remodeling function of iPLA$_2\beta$ in mast cells, because the phospholipid composition in *Pla2g6*$^{-/-}$ BMMCs was identical to that in wild-type BMMCs before and after coculture with fibroblasts. Dissociation of iPLA$_2\beta$ from phospholipid remodeling has also been demonstrated recently in testis, macrophages, and pancreatic β-cells (44, 76, 77), in which the enzyme appears to play signaling roles in processes

such as Ca^{2+} entry, lipid mediator synthesis, cell migration, exocytosis, and apoptosis (41–49). However, in BMMCs, iPLA$_2\beta$ is apparently nonessential for stimulus-coupled AA mobilization and subsequent eicosanoid generation, granule exocytosis, cytokine secretion, and even upstream Ca^{2+} entry (Figs. 2 and 3). Indeed, the normal PCA reaction in *Pla2g6*$^{-/-}$ mice implies that mast cells are numerically and functionally intact in the absence of iPLA$_2\beta$ *in vivo*. This argues against the proposed idea that iPLA$_2\beta$-released lysophospholipid is a prerequisite for opening of the Orai1-Stim1 CRAC channel (41), given that perturbation of this Ca^{2+}-sensitive Ca^{2+} channel profoundly hampers multiple effector functions of FcεRI-activated mast cells (61). Also, the proposed role of iPLA$_2\beta$ in the inflammasome-dependent IL-1β production, an idea that arose from the pharmacological inhibition exerted by BEL (45), is also unlikely in BMMCs, because our preliminary data show that IL-1β release was unaffected by iPLA$_2\beta$ deficiency (data not shown), and a similar result has recently been obtained by another group (63). Thus far, we have been unable to characterize any functional role of iPLA$_2\beta$ in mast cells, even though it is undeniably present.

Despite the unaltered effector functions of *Pla2g6*$^{-/-}$ mast cells, BEL potently inhibited β-HEX release by BMMCs (Fig. 1*D*), indicating that BEL acts on other target(s) that are crucial for mast cell exocytosis. Because nine iPLA$_2$ homologs, known as patatin-like phospholipase A domain-containing lipases (PNPLAs), are encoded in the mammalian genome (31, 78), it remains possible that the genetic deficiency of iPLA$_2\beta$ in mast cells might be compensated by other iPLA$_2$/PNPLA isoform(s) or that other BEL-sensitive iPLA$_2$/PNPLA isoform(s), rather than iPLA$_2\beta$, might truly contribute to the functions and/or maturation of mast cells. The possibility that iPLA$_2\beta$ deficiency is compensated by iPLA$_2\gamma$/PNPLA8 is unlikely, because there was no compensatory up-regulation of iPLA$_2\gamma$ in *Pla2g6*$^{-/-}$ BMMCs (supplemental Fig. S1) and because our preliminary data have shown that *Pnpla8*$^{-/-}$ BMMCs have normal degranulation capacity.[4] It has been recently shown that the lack of PNPLA2 (adipose triglyceride lipase or iPLA$_2\zeta$) impairs insulin secretion by pancreatic β-cells (79) and phagocytosis by macrophages (80) due to impaired lipolytic supply of fatty acid fuel. In yeast, Sec14p, a component of the secretory machinery, is functionally coupled with phospholipid deacylation by Nte1p, a yeast homolog of PNPLA6 (neuronal target esterase or iPLA$_2\delta$) (81). In analogy, mast cell degranulation might require energy supply by iPLA$_2\zeta$/PNPLA2 or vesicular trafficking by iPLA$_2\delta$/PNPLA6, a possibility that should await future studies. Note that BEL could also inhibit several enzymes unrelated to the iPLA$_2$/PNPLA family (62, 63). Thus, caution should be exercised when using this drug to study possible roles of iPLA$_2$s in cellular responses.

Several studies have reported the critical role of iPLA$_2\beta$ in lipid mediator synthesis by several cell types. For instance, thapsigargin- or A23187-stimulated AA release is dramatically attenuated in *Pla2g6*$^{-/-}$ aortic smooth muscle cells, and these cells show decreased migration and proliferation due to reduced PGE_2 generation in a model of vascular injury (42). A high glucose-induced iPLA$_2\beta$ activates RhoA/Rho kinase via 12/15-lipoxygenase metabolites, which contributes to vascular smooth muscle hypercontractility in diabetic animals (43). In lung endothelial cells, thrombin- or tryptase-stimulated synthesis of PGI_2 and platelet-activating factor is attenuated by iPLA$_2\beta$ deficiency (83). Even in nonvascular cells, scavenger receptor A-dependent adhesion, a process that requires 12/15-lipoxygenase but not COX products, is absent in *Pla2g6*$^{-/-}$ macrophages (84). Moreover, A23187-induced, but not IL-1β-stimulated, AA release was augmented in iPLA$_2\beta$-transfected HEK293 cells (70). We have shown here that the PGE_2 biosynthetic responses of dendritic cells and macrophages to LPS, but not zymosan, are partially reduced by the deficiency of iPLA$_2\beta$, confirming the involvement of iPLA$_2\beta$ in the former situation (supplemental Fig. S2). Taken together, the requirement of iPLA$_2\beta$ for cell activation may depend on cell type, stimulus, and/or class of lipid mediators. Although precise molecular mechanisms underlying iPLA$_2\beta$ activation in distinct contexts should need further clarification, mast cells represent a clear example that cell activation is fully operative in the absence of iPLA$_2\beta$. Presumably, mast cells might be intrinsically devoid of certain unknown factor(s) essential for iPLA$_2\beta$ activation or the lack of iPLA$_2\beta$ might be bypassed by alternative mechanism(s).

It is intriguing, however, that during maturation of BMMCs toward CTMC-like cells, iPLA$_2\beta$ shows an increase of expression accompanied by a molecular mass shift from the full size into smaller forms over time (Fig. 1*C*). This would suggest that, in addition to transcriptional control of iPLA$_2\beta$ (Fig. 1*A*), its expression is regulated by proteolytic processing (48, 49, 59, 60) or by alternative splicing (82, 85). It has been reported that proteolytic cleavage of the N-terminal ankyrin repeats of iPLA$_2\beta$ by caspase-3 or unknown proteases occurs in apoptotic cells (48, 49) or even in nonapoptotic migrating cells (60) and agonist-treated islet β-cells (59), leading to activation of this enzyme. Because we did not detect any functional role of iPLA$_2\beta$ in BMMCs, the regulatory mechanisms responsible for the proteolytic processing of iPLA$_2\beta$ and its functional consequences in CTMC-like differentiating cells are still a subject of debate. During apoptosis, the truncated iPLA$_2\beta$ contributes to apoptotic changes in membranes, and the LPC released from apoptotic cells acts as a chemoattractant ("find-me" signal) for phagocytotic cells (48, 49). In this view, iPLA$_2\beta$ might play a role in the phagocytotic clearance of a population of mature mast cells that are destined for apoptosis under certain conditions, a possibility that will need to be examined in a future study.

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REFERENCES

1. Metcalfe, D. D., Baram, D., and Mekori, Y. A. (1997) *Physiol. Rev.* **77**, 1033–1079
2. Wong, G. W., Friend, D. S., and Stevens, R. L. (1999) in *Signal Transduction in Mast Cells and Basophils* (Razin, E., and Rivera, J., eds) pp. 39–53,

Springer-Verlag, New York
3. Kalesnikoff, J., and Galli, S. J. (2008) *Nat. Immunol.* **9,** 1215–1223
4. Galli, S. J., Grimbaldeston, M., and Tsai, M. (2008) *Nat. Rev. Immunol.* **8,** 478–486
5. Huang, E., Nocka, K., Beier, D. R., Chu, T. Y., Buck, J., Lahm, H. W., Wellner, D., Leder, P., and Besmer, P. (1990) *Cell* **63,** 225–233
6. Nocka, K., Buck, J., Levi, E., and Besmer, P. (1990) *EMBO J.* **9,** 3287–3294
7. Gurish, M. F., Tao, H., Abonia, J. P., Arya, A., Friend, D. S., Parker, C. M., and Austen, K. F. (2001) *J. Exp. Med.* **194,** 1243–1252
8. Abonia, J. P., Austen, K. F., Rollins, B. J., Joshi, S. K., Flavell, R. A., Kuziel, W. A., Koni, P. A., and Gurish, M. F. (2005) *Blood* **105,** 4308–4313
9. Levi-Schaffer, F., Austen, K. F., Gravallese, P. M., and Stevens, R. L. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83,** 6485–6488
10. Dayton, E. T., Pharr, P., Ogawa, M., Serafin, W. E., Austen, K. F., Levi-Schaffer, F., and Stevens, R. L. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85,** 569–572
11. Ogasawara, T., Murakami, M., Suzuki-Nishimura, T., Uchida, M. K., and Kudo, I. (1997) *J. Immunol.* **158,** 393–404
12. Takano, H., Nakazawa, S., Okuno, Y., Shirata, N., Tsuchiya, S., Kainoh, T., Takamatsu, S., Furuta, K., Taketomi, Y., Naito, Y., Takematsu, H., Kozutsumi, Y., Tsujimoto, G., Murakami, M., Kudo, I., Ichikawa, A., Nakayama, K., Sugimoto, Y., and Tanaka, S. (2008) *FEBS Lett.* **582,** 1444–1450
13. Taketomi, Y., Sugiki, T., Saito, T., Ishii, S., Hisada, M., Suzuki-Nishimura, T., Uchida, M. K., Moon, T. C., Chang, H. W., Natori, Y., Miyazawa, S., Kikuchi-Yanoshita, R., Murakami, M., and Kudo, I. (2003) *Biochem. Biophys. Res. Commun.* **306,** 339–346
14. Taketomi, Y., Sunaga, K., Tanaka, S., Nakamura, M., Arata, S., Okuda, T., Moon, T. C., Chang, H. W., Sugimoto, Y., Kokame, K., Miyata, T., Murakami, M., and Kudo, I. (2007) *J. Immunol.* **178,** 7042–7053
15. Ruoss, S. J., Hartmann, T., and Caughey, G. H. (1991) *J. Clin. Invest.* **88,** 493–499
16. Levi-Schaffer, F., and Rubinchik, E. (1995) *J. Invest. Dermatol.* **104,** 999–1003
17. Kendall, J. C., Li, X. H., Galli, S. J., and Gordon, J. R. (1997) *J. Allergy Clin. Immunol.* **99,** 113–123
18. Trautmann, A., Krohne, G., Bröcker, E. B., and Klein, C. E. (1998) *J. Immunol.* **160,** 5053–5057
19. Akers, I. A., Parsons, M., Hill, M. R., Hollenberg, M. D., Sanjar, S., Laurent, G. J., and McAnulty, R. J. (2000) *Am. J. Physiol. Lung Cell. Mol. Physiol.* **278,** L193–L201
20. Smith, T. J., and Parikh, S. J. (1999) *Endocrinology* **140,** 3518–3525
21. Smith, R. S., Smith, T. J., Blieden, T. M., and Phipps, R. P. (1997) *Am. J. Pathol.* **151,** 317–322
22. Murray, J. J., Tonnel, A. B., Brash, A. R., Roberts, L. J., 2nd, Gosset, P., Workman, R., Capron, A., and Oates, J. A. (1986) *N. Engl. J. Med.* **315,** 800–804
23. Austen, K. F. (2008) *Nat. Immunol.* **9,** 113–115
24. Tager, A. M., Bromley, S. K., Medoff, B. D., Islam, S. A., Bercury, S. D., Friedrich, E. B., Carafone, A. D., Gerszten, R. E., and Luster, A. D. (2003) *Nat. Immunol.* **4,** 982–990
25. Lewis, R. A., Soter, N. A., Diamond, P. T., Austen, K. F., Oates, J. A., and Roberts, L. J., 2nd (1982) *J. Immunol.* **129,** 1627–1631
26. Murakami, M., Matsumoto, R., Urade, Y., Austen, K. F., and Arm, J. P. (1995) *J. Biol. Chem.* **270,** 3239–3246
27. Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K., Sugimoto, Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y., Yoshida, N., Kimura, K., Mizoguchi, A., Honda, Y., Nagai, H., and Narumiya, S. (2000) *Science* **287,** 2013–2017
28. Satoh, T., Moroi, R., Aritake, K., Urade, Y., Kanai, Y., Sumi, K., Yokozeki, H, Hirai, H., Nagata, K., Hara, T., Utsuyama, M., Hirokawa, K., Sugamura, K., Nishioka, K., and Nakamura, M. (2006) *J. Immunol.* **177,** 2621–2629
29. Hammad, H., Kool, M., Soullié, T., Narumiya, S., Trottein, F., Hoogsteden, H. C., and Lambrecht, B. N. (2007) *J. Exp. Med.* **204,** 357–367
30. Shiraishi, Y., Asano, K., Niimi, K., Fukunaga, K., Wakaki, M., Kagyo, J., Takihara, T., Ueda, S., Nakajima, T., Oguma, T., Suzuki, Y., Shiomi, T., Sayama, K., Kagawa, S., Ikeda, E., Hirai, H., Nagata, K., Nakamura, M., Miyasho, T., and Ishizaka, A. (2008) *J. Immunol.* **180,** 541–549
31. Kunikata, T., Yamane, H., Segi, E., Matsuoka, T., Sugimoto, Y., Tanaka, S., Tanaka, H., Nagai, H., Ichikawa, A., and Narumiya, S. (2005) *Nat. Immunol.* **6,** 524–531
32. Lundequist, A., Nallamshetty, S. N., Xing, W., Feng, C., Laidlaw, T. M., Uematsu, S., Akira, S., and Boyce, J. A. (2010) *J. Immunol.* **184,** 433–441
33. Kudo, I., and Murakami, M. (2002) *Prostaglandins Other Lipid Mediat.* **68,** 3–58
34. Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T., and Yamamoto, K. (2011) *Prog. Lipid Res.* **50,** 152–192
35. Fujishima, H., Sanchez Mejia, R. O., Bingham, C. O., 3rd, Lam, B. K., Sapirstein, A., Bonventre, J. V., Austen, K. F., and Arm, J. P. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96,** 4803–4807
36. Nakatani, N., Uozumi, N., Kume, K., Murakami, M., Kudo, I., and Shimizu, T. (2000) *Biochem. J.* **352,** 311–317
37. Kikawada, E., Bonventre, J. V., and Arm, J. P. (2007) *Blood* **110,** 561–567
38. Reddy, S. T., and Herschman, H. R. (1996) *J. Biol. Chem.* **271,** 186–191
39. Fensome-Green, A., Stannard, N., Li, M., Bolsover, S., and Cockcroft, S. (2007) *Cell Calcium* **41,** 145–153
40. Balsinde, J., Balboa, M. A., and Dennis, E. A. (1997) *J. Biol. Chem.* **272,** 29317–29321
41. Smani, T., Zakharov, S. I., Csutora, P., Leno, E., Trepakova, E. S., and Bolotina, V. M. (2004) *Nat. Cell Biol.* **6,** 113–120
42. Moon, S. H., Jenkins, C. M., Mancuso, D. J., Turk, J., and Gross, R. W. (2008) *J. Biol. Chem.* **283,** 33975–33987
43. Xie, Z., Gong, M. C., Su, W., Xie, D., Turk, J., and Guo, Z. (2010) *J. Biol. Chem.* **285,** 8628–8638
44. Bao, S., Song, H., Wohltmann, M., Ramanadham, S., Jin, W., Bohrer, A., and Turk, J. (2006) *J. Biol. Chem.* **281,** 20958–20973
45. Piccini, A., Carta, S., Tassi, S., Lasiglié, D., Fossati, G., and Rubartelli, A. (2008) *Proc. Natl. Acad. Sci. U.S.A.* **105,** 8067–8072
46. Mishra, R. S., Carnevale, K. A., and Cathcart, M. K. (2008) *J. Exp. Med.* **205,** 347–359
47. Dietrich, H. H., Abendschein, D. R., Moon, S. H., Nayeb-Hashemi, N., Mancuso, D. J., Jenkins, C. M., Kaltenbronn, K. M., Blumer, K. J., Turk, J., and Gross, R. W. (2010) *Am. J. Physiol. Heart Circ. Physiol.* **298,** H2208–H2220
48. Atsumi, G., Murakami, M., Kojima, K., Hadano, A., Tajima, M., and Kudo, I. (2000) *J. Biol. Chem.* **275,** 18248–18258
49. Lauber, K., Bohn, E., Kröber, S. M., Xiao, Y. J., Blumenthal, S. G., Lindemann, R. K., Marini, P., Wiedig, C., Zobywalski, A., Baksh, S., Xu, Y., Autenrieth, I. B., Schulze-Osthoff, K., Belka, C., Stuhler, G., and Wesselborg, S. (2003) *Cell* **113,** 717–730
50. Li, H., Zhao, Z., Wei, G., Yan, L., Wang, D., Zhang, H., Sandusky, G. E., Turk, J., and Xu, Y. (2010) *FASEB J.* **24,** 4103–4116
51. Morgan, N. V., Westaway, S. K., Morton, J. E., Gregory, A., Gissen, P., Sonek, S., Cangul, H., Coryell, J., Canham, N., Nardocci, N., Zorzi, G., Pasha, S., Rodriguez, D., Desguerre, I., Mubaidin, A., Bertini, E., Trembath, R. C., Simonati, A., Schanen, C., Johnson, C. A., Levinson, B., Woods, C. G., Wilmot, B., Kramer, P., Gitschier, J., Maher, E. R., and Hayflick, S. J. (2006) *Nat. Genet.* **38,** 752–754
52. Shinzawa, K., Sumi, H., Ikawa, M., Matsuoka, Y., Okabe, M., Sakoda, S., and Tsujimoto, Y. (2008) *J. Neurosci.* **28,** 2212–2220
53. Kamei, D., Yamakawa, K., Takegoshi, Y., Mikami-Nakanishi, M., Nakatani, Y., Oh-Ishi, S., Yasui, H., Azuma, Y., Hirasawa, N., Ohuchi, K., Kawaguchi, H., Ishikawa, Y., Ishii, T., Uematsu, S., Akira, S., Murakami, M., and Kudo, I. (2004) *J. Biol. Chem.* **279,** 33684–33695
54. Jenkins, C. M., Han, X., Mancuso, D. J., and Gross, R. W. (2002) *J. Biol. Chem.* **277,** 32807–32814
55. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917
56. Houjou, T., Yamatani, K., Imagawa, M., Shimizu, T., and Taguchi, R. (2005) *Rapid Commun. Mass Spectrom.* **19,** 654–666
57. Taguchi, R., Houjou, T., Nakanishi, H., Yamazaki, T., Ishida, M., Imagawa, M., and Shimizu, T. (2005) *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **823,** 26–36
58. Gurish, M. F., Ghildyal, N., McNeil, H. P., Austen, K. F., Gillis, S., and Stevens, R. L. (1992) *J. Exp. Med.* **175,** 1003–1012
59. Song, H., Bao, S., Lei, X., Jin, C., Zhang, S., Turk, J., and Ramanadham, S. (2010) *Biochim. Biophys. Acta* **1801,** 547–558
60. Zhao, X., Wang, D., Zhao, Z., Xiao, Y., Sengupta, S., Xiao, Y., Zhang, R.,

Lauber, K., Wesselborg, S., Feng, L., Rose, T. M., Shen, Y., Zhang, J., Prestwich, G., and Xu, Y. (2006) *J. Biol. Chem.* **281,** 29357–29368
61. Vig, M., DeHaven, W. I., Bird, G. S., Billingsley, J. M., Wang, H., Rao, P. E., Hutchings, A. B., Jouvin, M. H., Putney, J. W., and Kinet, J. P. (2008) *Nat. Immunol.* **9,** 89–96
62. Fuentes, L., Pérez, R., Nieto, M. L., Balsinde, J., and Balboa, M. A. (2003) *J. Biol. Chem.* **278,** 44683–44690
63. Franchi, L., Chen, G., Marina-Garcia, N., Abe, A., Qu, Y., Bao, S., Shayman, J. A., Turk, J., Dubyak, G. R., and Núñez, G. (2009) *J. Innate Immun.* **1,** 607–617
64. Murakami, M., Austen, K. F., and Arm, J. P. (1995) *J. Exp. Med.* **182,** 197–206
65. Clark, J. D., Lin, L. L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991) *Cell* **65,** 1043–1051
66. Hu, Z. Q., Asano, K., Seki, H., and Shimamura, T. (1995) *J. Immunol.* **155,** 2134–2142
67. Diaz, B. L., Fujishima, H., Kanaoka, Y., Urade, Y., and Arm, J. P. (2002) *J. Immunol.* **168,** 1397–1404
68. Gomi, K., Zhu, F. G., and Marshall, J. S. (2000) *J. Immunol.* **165,** 6545–6552
69. Nguyen, M., Solle, M., Audoly, L. P., Tilley, S. L., Stock, J. L., McNeish, J. D., Coffman, T. M., Dombrowicz, D., and Koller, B. H. (2002) *J. Immunol.* **169,** 4586–4593
70. Murakami, M., Kambe, T., Shimbara, S., and Kudo, I. (1999) *J. Biol. Chem.* **274,** 3103–3115
71. Wijewickrama, G. T., Kim, J. H., Kim, Y. J., Abraham, A., Oh, Y., Ananthanarayanan, B., Kwatia, M., Ackerman, S. J., and Cho, W. (2006) *J. Biol. Chem.* **281,** 10935–10944
72. Sala, A., Folco, G., and Murphy, R. C. (2010) *Pharmacol. Rep.* **62,** 503–510
73. Zarini, S., Gijón, M. A., Ransome, A. E., Murphy, R. C., and Sala, A. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106,** 8296–8301
74. Fabre, J. E., Goulet, J. L., Riche, E., Nguyen, M., Coggins, K., Offenbacher, S., and Koller, B. H. (2002) *J. Clin. Invest.* **109,** 1373–1380
75. Yamashita, A., Kamata, R., Kawagishi, N., Nakanishi, H., Suzuki, H., Sugiura, T., and Waku, K. (2005) *J. Biochem.* **137,** 557–567
76. Bao, S., Miller, D. J., Ma, Z., Wohltmann, M., Eng, G., Ramanadham, S., Moley, K., and Turk, J. (2004) *J. Biol. Chem.* **279,** 38194–38200
77. Bao, S., Bohrer, A., Ramanadham, S., Jin, W., Zhang, S., and Turk, J. (2006) *J. Biol. Chem.* **281,** 187–198
78. Kienesberger, P. C., Oberer, M., Lass, A., and Zechner, R. (2009) *J. Lipid Res.* **50,** S63–S68
79. Peyot, M. L., Guay, C., Latour, M. G., Lamontagne, J., Lussier, R., Pineda, M., Ruderman, N. B., Haemmerle, G., Zechner, R., Joly, E., Madiraju, S. R., Poitout, V., and Prentki, M. (2009) *J. Biol. Chem.* **284,** 16848–16859
80. Chandak, P. G., Radovic, B., Aflaki, E., Kolb, D., Buchebner, M., Fröhlich, E., Magnes, C., Sinner, F., Haemmerle, G., Zechner, R., Tabas, I., Levak-Frank, S., and Kratky, D. (2010) *J. Biol. Chem.* **285,** 20192–20201
81. Murray, J. P., and McMaster, C. R. (2005) *J. Biol. Chem.* **280,** 8544–8552
82. Larsson, P. K., Claesson, H. E., and Kennedy, B. P. (1998) *J. Biol. Chem.* **273,** 207–214
83. Sharma, J., Turk, J., and McHowat, J. (2010) *Biochemistry* **49,** 5473–5481
84. Nikolic, D. M., Gong, M. C., Turk, J., and Post, S. R. (2007) *J. Biol. Chem.* **282,** 33405–33411
85. Manguikian, A. D., and Barbour, S. E. (2004) *J. Biol. Chem.* **279,** 52881–52892