Microsomal Prostaglandin E Synthase-1 Deficiency Is Associated with Elevated PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ
REGULATION BY PROSTAGLANDIN E₂ VIA THE PHOSPHATIDYLINOSITOL 3-KINASE AND AKT PATHWAY

Mohit Kapoor, Fumiaki Kojima, Min Qian, Lihua Yang, and Leslie J. Crofford

From the *Department of Internal Medicine, Rheumatology Division, University of Kentucky, Lexington, Kentucky 40536 and the ‡Department of Ophthalmology and Visual Sciences, Division of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109

mPGES-1 (microsomal PGE synthase-1) is an inducible enzyme that acts downstream of cyclooxygenase (COX) and specifically catalyzes the conversion of prostaglandin (PG) H₂ to PGE₂ under basal as well as inflammatory conditions. In this study, using mouse embryo fibroblasts (MEFs) isolated from mice genetically deficient for the mPges-1 gene, we show basal elevation of peroxisome proliferator-activated receptor γ (PPARγ) expression (protein and mRNA) and transcriptional activity associated with reduced basal PGE₂. We further show that basal mPGES-1-derived PGE₂ suppresses the expression of PPARγ through a CAMP-independent pathway involving phosphatidylinositol 3-kinase and Akt signaling. Using specific PPARγ agonist (rosiglitazone), PPARγ ligand (15-deoxy-D12,14-PGF₂α), and PPARγ inhibitor (GW9662), we confirm that activation of PPARγ blocks interleukin-1β-induced up-regulation of COX-2, mPGES-1, and their derived PGE₂. Furthermore, we demonstrate that up-regulation of PPARγ upon genetic deletion of mPGES-1 is responsible for reduced COX-2 expression under basal as well as interleukin-1β-stimulated conditions. This study provides evidence for the first time that mPGES-1 deletion not only decreases proinflammatory PGE₂ but also up-regulates anti-inflammatory PPARγ, which has the ability to suppress COX-2 and mPGES-1 expression and PGE₂ production. Thus, mPGES-1 inhibition may limit inflammation by multiple mechanisms and is a potential therapeutic target.

Prostaglandins (PGs) are formed by metabolism of arachidonic acid by cyclooxygenase (COX) to generate an intermediate substrate, PGH₂, which is further metabolized by terminal synthases to generate specific PGs (1, 2). mPGES-1, originally known as microsomal glutathione S-transferase 1-like 1 (MGST1-L1), is an inducible enzyme that acts downstream of COX and specifically catalyzes the conversion of PGH₂ to PGE₂ (3), most prominently in inflammatory conditions (4, 5). However, we have recently shown that mPGES-1 is critical for PGE₂ production under basal as well as inflammatory conditions (6). mPGES-1 is coordinately induced with COX-2 by inflammatory stimuli in a variety of cells and tissues (4, 7). PGE₂ is the most abundant PG associated with inflammatory conditions, and overproduction of PGE₂ coincides with increased COX-2 and mPGES-1 expression (4, 7). PGE₂ exerts the majority of its actions through a family of G protein-coupled receptors, including EP1, EP2, EP3, and EP4 (8). The effects of PGE₂ via these receptors are mediated through various downstream signaling pathways, including cAMP-dependent protein kinase, mitogen-activated protein kinase (MAP kinase), phosphatidylinositol 3-kinase (PI 3-kinase), and Akt (8–10).

Inhibition of PGE₂ production and action is associated with reduction of the pain and inflammation associated with a wide variety of diseases. Nonselective and COX-2-selective nonsteroidal anti-inflammatory drugs (NSAIDs) block PGE₂ production by inhibiting the activity of COX and are extensively used to treat arthritis and other inflammatory conditions. However, side effects associated with the inhibition of COX-2 (11–13) have revived efforts to develop safer anti-inflammatory drugs. mPGES-1 is an attractive target to achieve more specific inhibition of PGE₂ production associated with inflammatory disorders while preserving production of other PGs. Specific inhibitors of mPGES-1 are yet not available; however, studies using mice genetically deficient in mPGES-1 have demonstrated that this enzyme is a key mediator of inflammation, pain, angiogenesis, fever, bone metabolism, and tumorigenesis (14–17). Our studies also demonstrate that mPGES-1 expression is increased in tissues and cells of various inflammatory conditions, including rheumatoid arthritis and osteoarthritis (4, 5, 18, 19). Previs-
uous studies have also shown that mPGES-1 null mice are resistant to arthritis in the models of collagen-induced arthritis and collagen antibody-induced arthritis (14, 15).

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of nuclear hormone receptor superfamily of ligand-activated transcription factors that have been shown to regulate inflammatory responses and assist in the resolution of inflammation (20–24). Recent studies have shown a close relationship between PPARγ and PGs in the regulation of inflammation (25–27). However, until now, no study has evaluated the potential role of mPGES-1 in the regulation of PPARγ. We created mouse embryo fibroblast (MEF) cell lines derived from mPGES-1 null mice and wild type (WT) littermates to facilitate these studies. This study demonstrates for the first time that mPGES-1 deficiency and reduced PGE2 lead to elevation of downstream signaling targets responsible for PPARγ regulation by mPGES-1-derived PGE2.

EXPERIMENTAL PROCEDURES

Animals—mPGES-1 heterozygous mice on a DBA1 lac/J background were obtained from Pfizer (15). Mice were housed in microisolator cages in a pathogen-free barrier facility, and all background were obtained from Pfizer (15). Mice were housed down-stream signaling targets responsible for PPAR

Measurement of PGE2 in Culture Medium—MEFs were incubated for 12 h in the presence or absence of IL-1β (1 ng/ml). In

PPARγ Transcriptional Activity Assay—MEFs were plated into the T-75 flasks at a density of 2 × 105 cells/flask in DMEM containing 10% FBS. Cells were starved for 72 h in DMEM containing 1% FBS and then incubated with or without 1 ng/ml IL-1β in the absence of various treatments for 12 h. Cell viability was determined by measuring mitochondrial NADH-dependent dehydrogenase activity with WST-1 assay (Dojindo Laboratories, Kumamoto, Japan).

Reverse Transcription (RT)-PCR—RNA from the cells was extracted with TRIpure reagent according to the manufacturer’s recommendations. Reverse transcription was performed according to the manufacturer’s instructions using a SuperScript pre-amplification system (Invitrogen) with 1 μg of total RNA from the cells as a template. Subsequent amplifications of the cDNA fragments by PCR with HotStar Taq polymerase (Qiagen, Valencia, CA) were performed using 0.5 μl of the reverse-transcribed mixture as a template with specific oligonucleotide primers and cycle numbers as follows: mouse PPARγ (31 cycles), sense 5’-CCT CTC CGT GAT GGA AGA CC-3’ and antisense 5’-GCA TTG TGA GAC ATC CCC AC-3’; mouse GAPDH (20 cycles), sense 5’-GGG GTG AGG CCC GTG CTG AGT AT-3’ and antisense 5’-CAT TGG GGG TGG TAG CAA GCC GAA AGG-3’. After initial denaturation at 95 °C for 15 min, PCR involved amplification cycles of 30 s at 95 °C, 30 s at 56 °C, and 4 s at 72 °C, followed by elongation for 5 min at 72 °C. The amplified cDNA fragments were resolved by electrophoresis on 2% (w/v) agarose gel and were visualized under UV light using a Chemidoc apparatus (Bio-Rad) after staining of the gel with ethidium bromide.

Western Blotting—Cells were lysed in Tris-buffered saline (TBS) containing 0.1% SDS, and the protein content of the lysates was determined using bicinchoninic acid (BCA) protein assay reagent (Pierce) with bovine serum albumin as the standard. Cell lysates were adjusted to equal equivalents of protein and then were applied to SDS-polyacrylamide gels (10–20%) for electrophoresis. Next, the proteins were electroblotted onto polyvinylidene difluoride membranes. After the membranes were blocked in 10 mM TBS containing 0.1% Tween 20 (TBS-T) and 5% skim milk, the membranes were probed for 1.5 h with the respective antibodies (1:1000 for mPGES-1, COX-2, PPARγ, and β-actin) in TBS-T for 1.5 h. After washing the membranes with TBS-T, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit (for mPGES-1 and COX-2) or horseradish peroxidase-conjugated anti-mouse IgG were obtained from Jackson ImmunoResearch. Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were from Invitrogen. Recombinant mouse IL-1β was obtained from R&D Systems (Minneapolis, MN). TRIPure was purchased from Roche Diagnostics. The polyvinylidene difluoride membrane and enhanced chemiluminescence (ECL) reagent were purchased from Amersham Biosciences.

Preparation and Activation of Mouse Embryo Fibroblasts—Embryos were harvested from mPGES-1 (DBA1 lac/l) pregnant heterozygous females (E12.5) who had been mated with heterozygous males. Whole embryos were minced and placed into culture DMEM containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C under an atmosphere of 5% CO2. At confluence, the cells were detached and passed, and 3–4 passage cells were used in all experiments. MEFs were plated into the wells of a 6-well plate at a density of 3 × 105 cells/well in DMEM containing 10% FBS. Cells were starved for 72 h in DMEM containing 1% FBS and then incubated with or without 1 ng/ml IL-1β in the presence or absence of various treatments for 12 h. Cell viability was determined by measuring mitochondrial NADH-dependent dehydrogenase activity with WST-1 assay (Dojindo Laboratories, Kumamoto, Japan).
experiments involving treatment with indomethacin and NS-398, these compounds were added 72 h before IL-1/ H9252 stimulation. The culture supernatant was harvested, and concentration of PGE2 was measured by ELISA (Cayman Chemical, Ann Arbor MI). Assays were performed according to the manufacturer’s recommendation.

**Statistical Analysis**—The data are expressed as mean ± S.E. Statistical analysis was performed using Student’s *t* test. *p* < 0.05 was considered statistically significant.

**RESULTS**

**Effect of mPGES-1 Genetic Deletion on PPARγ Expression (mRNA and Protein) and Transcriptional Activity in IL-1β-stimulated and unstimulated MEFs**—mPGES-1 WT and null MEFs were incubated with or without IL-1β (1 ng/ml) for 12 h. α, mRNA levels of PPARγ (30 cycles) and GAPDH (20 cycles) from mPGES-1 WT and null MEFs were determined by RT-PCR. Mean ± S.E. for PPARγ expression normalized with GAPDH from n = 3 separate embryo lines is shown. *, mPGES-1 null MEFs compared with mPGES-1 WT MEFs. Cont, control. b, PPARγ and β-actin protein expressions were determined by Western blotting. Mean ± S.E. for PPARγ expression normalized with β-actin from n = 5 separate embryo lines is shown. *, mPGES-1 null MEFs compared with mPGES-1 WT MEFs. c, PPARγ transcriptional activity was measured in the nuclear extracts of IL-1β-stimulated and unstimulated mPGES-1 WT and null MEFs. Open bars represent mPGES-1 WT MEFs, and closed bars represent mPGES-1 null MEFs. PPARγ transcriptional activity was normalized to mg of protein for each sample. Data are expressed as the mean ± S.E. for four embryo lines. * and ** indicate statistical significance at *p* < 0.05 and *p* < 0.01, respectively.

**Effect of mPGES-1 Genetic Deletion on PGE2 Levels in mPGES-1 WT and Null MEFs**—PGE2 levels were significantly (*p* < 0.05) higher in mPGES-1 WT MEFs compared with null MEFs under unstimulated conditions (Fig. 2). Upon stimulation with IL-1β, a significant (*p* < 0.01) increase in the levels of PGE2 was observed in WT MEFs; however, no change in the PGE2 levels was observed in mPGES-1 null MEFs. These results clearly demonstrate that mPGES-1 is critical for PGE2 production at basal as well as stimulated conditions even though we have shown previously that both cytosolic PGES (cPGES) and mPGES-2 are expressed in these MEFs (6).

Our recent study using mPGES-1 WT and null MEFs also showed that genetic deletion of mPGES-1 not only blocks PGE2 production but also results in the elevation of 6-keto-PGF1α (stable breakdown product of prostacyclin; PGI2) under basal conditions.
well as cytokine-stimulated conditions, suggesting a shunting phenomenon within the arachidonic acid metabolic pathway upon deletion of mPGES-1 (6). In this study, we further showed that mPGES-1 genetic deletion did not have any effect on the production pattern of other PGs such as PGD₂ and thromboxane B₂, which remained unaltered in mPGES-1 WT and null MEFs under basal as well as cytokine-stimulated conditions.

**Effect of Exogenous PGE₂ and Carbachol (Prostacyclin Analogue) on PPARγ Expression and Transcriptional Activity in mPGES-1 WT and Null MEFs**—To determine whether PGE₂ or an alternate change induced by mPGES-1 deletion suppresses PPARγ expression and transcriptional activity in MEFs, exogenous PGE₂ treatment of mPGES-1 WT and null MEFs in the presence or absence of IL-1β stimulation was performed. Addition of PGE₂ under basal conditions resulted in a significant decrease (p < 0.05) in the PPARγ expression both at mRNA and protein levels in mPGES-1 null MEFs with little or no change observed in WT MEFs (Fig. 3, a and b). In the presence of IL-1β, a further decrease in the PPARγ mRNA expression levels was observed in both mPGES-1 WT and null MEFs. PPARγ protein expression did not show any further decrease when PGE₂ was used in combination with IL-1β.

Because our previous studies using mPGES-1 WT and null MEFs showed elevation of 6-keto-PGF₁α upon genetic deletion of mPGES-1 in MEFs (6), we therefore investigated the effects of carbachol (PGI₁ analogue) to determine the contribution of PGI₁ versus PGE₂ toward regulation of PPARγ expression in MEFs. However, we did not observe any changes in the protein expression levels of PPARγ in mPGES-1 WT and null MEFs upon treatment with carbachol (Fig. 3c).

Addition of PGE₂ in the absence of IL-1β stimulation significantly (p < 0.01) decreased PPARγ transcriptional activity levels in mPGES-1 null MEFs bringing the levels similar to mPGES-1 WT MEFs (Fig. 3d). In the presence of IL-1β stimulation, a further significant decrease in the levels of both mPGES-1 WT (p < 0.05) and null MEFs (p < 0.01) was observed. Treatment with carbachol did not have any effect on PPARγ transcriptional activity in mPGES-1 WT and null MEFs. These results suggest that deletion of mPGES-1 and a subsequent decrease in PGE₂ levels (and not increased PGI₁) play a key role in the differential regulation of PPARγ in MEFs. In addition, these results also suggest that IL-1β has the ability to itself regulate the expression and transcriptional activity of PPARγ and may exert other intrinsic effects on PPARγ expression and activity in addition to its ability to increase PGE₂ production.

**Effect of PGE₂ Inhibition by NSAIDs (Indomethacin and NS-398) on PPARγ Expression and Transcriptional Activity in mPGES-1 WT and Null MEFs**—To further confirm the contribution of PGE₂ toward differential regulation of PPARγ expression and transcriptional activity, mPGES-1 WT and null MEFs were treated with NSAIDs, including indomethacin (nonselective COX inhibitor) and NS-398 (selective COX-2 inhibitor). Addition of indomethacin and NS-398 significantly increased the levels of PPARγ protein expression (Fig. 4a) and transcriptional activity (Fig. 4b) in mPGES-1 WT MEFs only, raising the levels similar to that of mPGES-1 null MEFs. These results further confirm that PGE₂ is the key mediator involved in differential regulation of PPARγ in mPGES-1 WT and null MEFs.

**Effect of mPGES-1 Deletion on cAMP Levels in mPGES-1 WT and Null MEFs**—PGE₂ has been shown to mediate some of its downstream effects via cAMP-dependent pathways (8). Therefore, we investigated the effect of mPGES-1 deletion and the subsequent decrease in the levels of PGE₂ on cAMP levels in mPGES-1 WT and null MEFs. Results showed that mPGES-1 deletion did not have any significant effect on the levels of cAMP in MEFs under basal conditions (Fig. 5a). We further investigated the effects of exogenous PGE₂ and forskolin (a direct adenyl cyclase activator) on the levels of cAMP in mPGES-1 WT and null MEFs. Treatment with PGE₂ (p < 0.05) and forskolin (p < 0.01) significantly increased cAMP levels in both mPGES-1 WT and null MEFs to a similar extent. These results show that the cAMP machinery is intact in both mPGES-1 WT and null MEFs.

**Effect of Forskolin on PPARγ Expression and Transcriptional Activity Levels in mPGES-1 WT and Null MEFs**—To delineate the mechanism by which PGE₂ regulates PPARγ expression and transcriptional activity, we treated mPGES-1 WT and null MEFs with forskolin in the presence/absence of PGE₂, and we assessed its effect on PPARγ protein expression and transcriptional activity. Forskolin did not have any effect on PPARγ protein expression (Fig. 5b) and transcriptional activity (Fig. 5c) in mPGES-1 WT and null MEFs in the absence of PGE₂. However, PPARγ protein expression and transcriptional activity were significantly (p < 0.01) decreased when forskolin was used in combination with PGE₂, an effect that seems to be solely elicited by PGE₂ and not forskolin. These results suggest that even though cAMP pathways are activated by PGE₂ and forskolin in MEFs, PGE₂ regulates PPARγ expression and transcriptional activity via a signaling pathway independent of cAMP.

**Effect of PI 3-Kinase Inhibitor, Akt Inhibitor, p38 Inhibitor, and MEK Inhibitor on PPARγ Expression in mPGES-1 WT and Null MEFs**—PGE₂ is also known to mediate some of its biological responses through MAP kinase and PI 3-kinase/Akt pathways (8). Therefore, to delineate the mechanism with which PGE₂ regulates PPARγ expression in mPGES-1 WT and null
FIGURE 3. Effect of PGE2 and carbacyclin on PPARγ expression (mRNA and protein) and transcriptional activity in mPGES-1 WT and null MEFs. mPGES-1 WT and null MEFs were incubated for 12 h with PGE2 (1 μM) or carbacyclin (1 μM) in the presence or absence of IL-1β (1 ng/ml). a, mRNA levels of PPARγ (30 cycles) and GAPDH (20 cycles) from mPGES-1 WT and null MEFs were determined by RT-PCR. Mean ± S.E. for PPARγ expression normalized with GAPDH from n = 3 separate embryo lines is shown. Cont, control; *, mPGES-1 null MEFs compared with mPGES-1 WT MEFs; †, IL-β-stimulated group compared with unstimulated controls; $, IL-β + PGE2-stimulated group compared with unstimulated controls. b, PPARγ and β-actin protein expressions were determined by Western blotting. Mean ± S.E. for PPARγ expression normalized with β-actin from n = 6 separate embryo lines is shown. *, mPGES-1 null MEFs compared with mPGES-1 WT MEFs; †, PGE2-treated group compared with respective untreated controls; $, IL-β + PGE2-stimulated group compared with respective unstimulated controls. c, PPARγ and β-actin protein expressions were determined by Western blotting. Mean ± S.E. for PPARγ expression normalized with β-actin from n = 3 separate embryo lines is shown. *, mPGES-1 null MEFs compared with mPGES-1 WT MEFs; †, PGE2-treated group compared with their respective untreated controls. d, PPARγ transcriptional activity was measured in the nuclear extracts of mPGES-1 WT and null MEFs treated with PGE2 or carbacyclin in the absence or presence of IL-1β. Open bars represent mPGES-1 WT MEFs, and closed bars represent mPGES-1 null MEFs. PPARγ transcriptional activity was normalized to mg of protein for each sample. Data are expressed as the mean ± S.E. for 3–4 embryo lines. * and ** indicate statistical significance at p < 0.05 and p < 0.01 respectively.
MEFs, we investigated the effects of LY294002 (specific PI 3-kinase inhibitor), 1L-6-hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecylcarbonate (specific Akt inhibitor), SB-203580 (selective p38 MAP kinase inhibitor), and PD98059 (selective MEK inhibitor) on PPARγ expression and transcriptional activity in mPGES-1 WT and null MEFs. Pretreatment with rosiglitazone and 15-deoxy-PGJ2 reduced COX-2 expression via the PPARγ pathway, we incubated mPGES-1 MEFs with rosiglitazone and 15-deoxy-PGJ2 in the presence or absence of PPARγ inhibitor (GW9662). GW9662 completely reversed the inhibitory effect of 15-deoxy-PGJ2 on COX-2 expression but showed only partial recovery on the inhibitory effect of 15-deoxy-PGJ2 on COX-2 expression in mPGES-1 WT and null MEFs. Some effects of 15-deoxy-PGJ2 have been shown previously to be mediated via alternate mechanistic pathways independent of PPARγ (26). In this study, partial recovery of COX-2 expression by GW9662 in the 15-deoxy-PGJ2 group further suggests the involvement of an alternative mechanistic pathway in addition to the PPARγ pathway by which 15-deoxy-PGJ2 blocks COX-2 expression in MEFs.

We further investigated the effects of rosiglitazone and 15-deoxy-PGJ2 on mPGES-1 expression in mPGES-1 WT MEFs. Low level of mPGES-1 protein was observed in unstimulated mPGES-1 WT MEFs (Fig. 7a). Upon stimulation with IL-1β, a significant elevation (p < 0.05) in the protein expression of COX-2 was observed. Pretreatment with rosiglitazone and 15-deoxy-PGJ2 blocked up-regulated expression of COX-2 in IL-1β-stimulated mPGES-1 WT. To confirm that rosiglitazone and 15-deoxy-PGJ2 reduced COX-2 expression via the PPARγ pathway, we incubated mPGES-1 MEFs with rosiglitazone and 15-deoxy-PGJ2 in the presence or absence of PPARγ inhibitor (GW9662). GW9662 completely reversed the inhibitory effect of 15-deoxy-PGJ2 on COX-2 expression but showed only partial recovery on the inhibitory effect of 15-deoxy-PGJ2 on COX-2 expression in mPGES-1 WT and null MEFs. Some effects of 15-deoxy-PGJ2 have been shown previously to be mediated via alternate mechanistic pathways independent of PPARγ (26). In this study, partial recovery of COX-2 expression by GW9662 in the 15-deoxy-PGJ2 group further suggests the involvement of an alternative mechanistic pathway in addition to the PPARγ pathway by which 15-deoxy-PGJ2 blocks COX-2 expression in MEFs.
in IL-1β-stimulated mPGES-1 WT and null MEFs. To confirm that rosiglitazone and 15-deoxy-PGJ₂ reduced mPGES-1 expression via the PPARγ pathway, we incubated mPGES-1 WT MEFs with rosiglitazone and 15-deoxy-PGJ₂ in the presence or absence of PPARγ inhibitor (GW9662). GW9662 significantly \( p < 0.05 \) reversed the inhibitory effects of rosiglitazone and 15-deoxy-PGJ₂ on mPGES-1 expression. These results show that increased PPARγ expression results in down-regulation of COX-2 and mPGES-1 expression under basal as well as inflammatory conditions.

A significant elevation \( p < 0.05 \) in the levels of PGE₂ was observed in MEFs upon stimulation with IL-1β compared with unstimulated MEFs (Fig. 7b). Treatment with rosiglitazone and 15-deoxy-PGJ₂ significantly \( p < 0.05 \) blocked the increased production of PGE₂ in IL-1β-stimulated mPGES-1 WT MEFs. This inhibition was recovered when rosiglitazone and 15-deoxy-PGJ₂ were used in the presence of GW9662 in IL-1β-stimulated mPGES-1 WT MEFs. These results show that increased PPARγ expression results in decreased PGE₂ production under basal as well as inflammatory conditions.

Because our results showed that genetic deletion of mPGES-1 and resultant decrease in PGE₂ production increased the levels of PPARγ in MEFs, we expected that mPGES-1 null MEFs would have low levels of COX-2 compared with WT MEFs. Indeed, we observed significantly higher levels of COX-2 protein in mPGES-1 WT MEFs compared with null MEFs under basal conditions (Fig. 7c). These results suggest that increased PPARγ expression as a result of genetic deletion of mPGES-1 could lead to decreased COX-2 expression in mPGES-1 null MEFs under basal conditions.

**DISCUSSION**

This study using MEFs isolated from mPGES-1-deficient mice clearly presents three major conclusions. First, genetic deletion of mPGES-1 and resultant decrease in PGE₂ production increased the levels of PPARγ in MEFs, we expected that mPGES-1 null MEFs would have low levels of COX-2 compared with WT MEFs. Indeed, we observed significantly higher levels of COX-2 protein in mPGES-1 WT MEFs compared with null MEFs under basal conditions (Fig. 7c). These results suggest that increased PPARγ expression as a result of genetic deletion of mPGES-1 could lead to decreased COX-2 expression in mPGES-1 null MEFs under basal conditions.
IL-1β-induced up-regulation of proinflammatory COX-2, mPGES-1, and their derived PGE2 in WT MEFs, whereas increased PPARγ in mPGES-1 null MEFs is associated with decreased COX-2 expression under basal conditions and after treatment with IL-1β.

**Differential Regulation of PPARγ in mPGES-1 Null MEFs**—mPGES-1 is an inducible enzyme that acts downstream of COX and specifically catalyzes the conversion of PGH2 to PGE2. Using MEFs isolated from mPGES-1-deficient mice, we have shown previously that in the absence of mPGES-1, low levels of PGE2 are produced under basal as well as cytokine-stimulated conditions (6). In this study, we demonstrate for the first time that genetic deletion of mPGES-1 leads to increased PPARγ expression and transcriptional activity in MEFs. Exogenous PGE2 decreased and NSAIDs (indomethacin, a nonselective COX inhibitor, and NS-398, a selective COX-2 inhibitor) increased PPARγ under basal conditions confirming that the presence of PGE2 down-regulates PPARγ, whereas PGE2 depletion results in the up-regulation of PPARγ.

PGE2 Regulates PPARγ Expression via Non-cAMP Pathway—Involving PI 3-Kinase and Akt Signaling—Genetic deletion of specific genes can be associated with unforeseen phenotypic changes. However, we show here that PGE2 is responsible for the differential regulation of PPARγ expression and transcriptional activity in MEFs. Exogenous PGE2 decreased PPARγ expression and transcriptional activity in MEFs. PGE2 exerts the majority of its actions through a family of G protein-coupled receptors, including EP1, EP2, EP3, and EP4 via downstream signaling pathways, including CAMP-dependent protein kinase, MAP kinase, and PI 3-kinase/Akt signaling (8, 30–32).

In this study we first confirmed that the cAMP pathway was intact in mPGES-1 WT and null MEFs. However, forskolin, a direct adenyl cyclase activator, did not lead to any change in PPARγ expression and transcriptional activity in either mPGES-1 WT or null MEFs in the presence or absence of PGE2. These data suggest that regulation of PPARγ by mPGES-1-derived PGE2 occurs via a CAMP-independent pathway. No changes in PPARγ expression were observed following inhibition of MEK or p38 MAP kinase. However, our results clearly show that PI 3-kinase and Akt inhibitors significantly up-regulated PPARγ expression in mPGES-1 WT MEFs, raising the levels similar to mPGES-1 null MEFs. PI 3-kinase and its immediate downstream effector Akt are therefore the key downstream signaling pathways by which PGE2 mediates its regulatory effects on various downstream targets via PI 3-Kinase and Akt signaling pathways (33, 34).

**Negative Regulation of Proinflammatory COX-2, mPGES-1, and Their Derived PGE2 by PPARγ**—PPARγ is an endogenous regulator known to mediate its anti-inflammatory effects by down-regulation of proinflammatory mediators (20, 21, 29). In contrast COX-2, mPGES-1, and their derived PGE2 are key proinflammatory mediators involved during initiation of inflam-
Using specific PPARγ agonist (rosiglitazone), PPARγ ligand (15-deoxy-PGJ2), and PPARγ inhibitor (GW9662), we observed that specific activation of PPARγ blocked IL-1β-induced up-regulation of proinflammatory COX-2, mPGES-1, and their derived PGE2 in WT MEFs. Because of the elevated PPARγ levels observed upon genetic deletion of mPGES-1, we therefore expected that mPGES-1 null MEFs would have lower levels of COX-2 compared with WT MEFs. Indeed, our results showed significantly lower levels of COX-2 protein in mPGES-1 null MEFs compared with WT MEFs under basal conditions. These results further confirm the endogenous anti-inflammatory properties of PPARγ in down-regulating key proinflammatory signals.

Significance of mPGES-1 Inhibition—PGE2 is a key proinflammatory mediator of inflammation associated with various disease states, and increased PGE2 requires both COX-2 and mPGES-1 (4, 5, 18, 19, 35–38). Nonselective and COX-2-selective NSAIDs reduce PGE2 production by inhibiting COX-2 activity and are extensively used for reducing inflammation, pain, and fever (39). COX-2-specific NSAIDs were developed with improved gastrointestinal safety (40–42). However, recent clinical trials using selective COX-2 inhibitors suggest that specific inhibition of COX-2 is associated with increased incidence of cardiovascular events (11–13). Specific COX-2 inhibition results in loss of anti-thrombotic prostacyclin (PGI2) derived from endothelial COX-2, which plays a key role in the regulation of thrombogenesis (43) and is a possible factor associated with cardiovascular side effects observed with the use of specific COX-2 inhibitors.

mPGES-1 specifically catalyzes the conversion of PGH2 to PGE2, particularly during inflammation, and is an attractive target to achieve more specific inhibition of PGE2 production (3). Recent studies by our group and others (6, 44, 45) have
shown that genetic deletion of mPGES-1 results in diversion of prostaglandin production from predominant PGE$_2$ toward PGI$_2$. In addition, we have recently shown that genetic deletion of mPGES-1 results in up-regulation of nitrite levels (stable metabolic product of nitric oxide (NO)) (6). PGI$_2$ and NO are key mediators involved in maintaining vascular homeostasis (46). A recent in vivo study by Fitzgerald and co-workers (45) also showed that mPGES-1 deletion depressed PGE$_2$ and increased PGI$_2$, with no effect on thrombogenesis or blood pressure in mice. Thus, these observations suggest that inhibition of mPGES-1 may avoid the cardiovascular side effects seen with inhibition of COX-2. In addition, this study shows that genetic deletion of mPGES-1 elevates anti-inflammatory PPAR$\gamma$. In vitro and in vivo studies suggest that PPAR$\gamma$ has the ability to stimulate anti-inflammatory responses and assist in the resolution of inflammation by inhibiting a broad range of proinflammatory mediators, including IL-1$\beta$, tumor necrosis factor-$\alpha$, and nuclear transcription factor $\kappa$B (20, 23, 28, 47). In this study we also demonstrate that PPAR$\gamma$ has the ability to down-regulate proinflammatory COX-2, mPGES-1, and their derived PGE$_2$ (Fig. 8).

Although the biology of mPGES-1 and the consequences of blocking its activity have yet to be completely delineated, mPGES-1 remains a viable target. It is not known if the efficacy of such a therapeutic strategy would equal inhibition of COX and all downstream PGs; however, data in mPGES-1 null mice and the efficacy of monoclonal antibodies against PGE$_2$ in an arthritis model (48) offer promise. Studies to date in mPGES-1 null mice and MEFs suggest that inhibiting mPGES-1 may be associated with downstream changes, such as increased NO (6) and PPAR$\gamma$, which would promote efficacy and potentially limit adverse effects associated with pharmacological inhibition of mPGES-1.

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