Deletion of Microsomal Prostaglandin E2 (PGE2) Synthase-1 Reduces Inducible and Basal PGE2 Production and Alters the Gastric Prostanoid Profile*

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Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible protein recently shown to be an important source of inflammatory PGE2. Here we have used mPGES-1 wild type, heterozygote, and null mice to assess the impact of reduction or absence of mPGES-1 protein on the production of PGE2 and other prostanoids in lipopolysaccharide (LPS)-treated macrophages and mice. Thiglycollate-elicited peritoneal macrophages with mPGES-1 deficiency were found to lose their ability to produce PGE2 upon LPS stimulation. Resident mPGES-1−/− peritoneal macrophages exhibited severely impaired PGE2-releasing activity but retained some LPS-inducible PGE2 production capacity. Both macrophage types showed a 50% decrease in PGE2 production with removal of one copy of the mPGES-1 gene. In vivo, mPGES-1 deletion abolished the LPS-stimulated production of PGE2 in spleen, kidney, and brain. Surprisingly, lack of mPGES-1 activity resulted in an 80–90% decrease in basal, cyclooxygenase-1 (COX-1)-dependent PGE2 production in stomach and spleen, and a 50% reduction in brain and kidney. Other prostanoids (thromboxane B2, PGD2, PGF2α, and 6-keto-PGF1α) were significantly elevated in stomachs of mPGES-1-null mice but not in other tissues. Examination of mRNA for several terminal prostaglandin synthase antibody product did not reveal changes in expression levels associated with mPGES-1 deficiency, indicating that gastric prostaglandin changes may be due to shunting of cyclooxygenase products to other terminal synthases. These data demonstrate for the first time a dual role for mPGES-1 in both inflammatory and COX-1-mediated PGE2 production and suggest an interdependence of prostanoid production with tissue-specific alterations of prostaglandin levels in the absence of mPGES-1.

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glossary:

Prostaglandins (PG)1 are lipid metabolites of arachidonic acid that are synthesized by a two-step reaction catalyzed by a cyclooxygenase and a terminal prostaglandin synthase. The cyclooxygenase product PGH2 serves as common precursor to all five major prostanoids (TXA2, PGE2, PGD2, PGI2, and PGF2α). The physiological roles of PG are both diverse and complex, with effects on kidney ion transport, vascular homeostasis, gastrointestinal protection and motility, pregnancy and parturition, sleep, and immune function (1–3). In particular, the primary mediators of pain and inflammation are PGE2 and PGI2 (4), whereas pyresis is mediated by PGE2 through the EP3 receptor (5).

Two major cyclooxygenase isoforms are known, each with distinct roles. Expression patterns suggest that the constitutively expressed COX-1 plays housekeeping functions, whereas the inducible COX-2 is implicated in inflammatory processes. Exceptions to this paradigm have been uncovered with observations of constitutive COX-2 expression in several neuronal structures of the brain and in the kidney (6–9). Thus, although COX-2 plays a pivotal role in inflammation, it also serves housekeeping functions. Indeed, the phenotype of the COX-2 null mice is indicative of COX-2 roles during kidney development, kidney homeostasis, ovulation, and parturition (10–13).

The terminal prostaglandin synthases vary widely in structure and distribution. PGE2 synthesis can be catalyzed by at least three different terminal prostaglandin synthases (14–19). As with COX-2, the expression of microsomal PGE synthase-1 (mPGES-1) is induced by cytokines and inflammatory stimuli in cultured cells (15, 18, 20–24) and in models of inflammation and fever in vivo (8, 17, 25–28). In contrast, the expression of cytosolic PGE synthase and microsomal PGE synthase-2 (mPGES-2) is not significantly induced by inflammatory stimuli (16, 28, 29). The μ-class glutathione S-transferases μ2 and μ3 also have the capacity to convert PGH2 to PGE2 (14).

The inducible nature of the mPGES-1 and COX-2 genes, together with studies of cotransfection of various COX and PGE synthase isoforms, suggest that COX-2 works more efficiently with mPGES-1, whereas COX-1 is enzymatically coupled preferentially with cytosolic prostaglandin E synthase (16, 20). mPGES-2 was shown to function equally well with both COX-1 and COX-2 (29). Hence, it is hypothesized that mPGES-1 acts as a source of inflammatory PGE2. In support of this, thioglycollate-elicited macrophages from mPGES-1-null mice are unable to produce PGE2 following LPS treatment (23), and mice lacking mPGES-1 show a strong reduction in both the incidence and severity of collagen-induced arthritis or LPS-induced fever (30, 31). Clearly, mPGES-1 plays an important role during inflammation in vivo. On the other hand, whether mPGES-1 contributes to basal PGE2 production is unknown, and the effect of eliminating mPGES-1 activity on the production of other prostanoids has never been assessed.

Here, we addressed these issues by examining the impact of
mPGES-1 and Gastric Prostaglandins

mPGES-1 deficiency on synthesis of major prostaglandins and thromboxane, both in cultured macrophages and in vivo. We find that mPGES-1 is necessary for PGE_{2} production, in a gene dosage-dependent manner, in resident and thioglycollate-elicited peritoneal macrophages. In vivo, we show that mPGES-1 is responsible for PGE_{2} elevation is several tissues of LPS-challenged mice, and in addition, contributes significantly to basal PGE_{2} levels in brain, kidney, stomach, and spleen. The stools of mPGES-1-null mice exhibited level alterations for all prostaglandins, without changes in mRNA expression of other terminal synthases. Taken together, these data uncover an unexpected contribution of mPGES-1 to basal, non-inflammatory PGE_{2} and an imbalance in other prostaglandins, most noticeable in the stomach of mice deficient for mPGES-1 activity.

**EXPERIMENTAL PROCEDURES**

Prostaglandins (PGs), deuterated PGs, and indomethacin were purchased from Cayman Chemical. Non-deuterated PGs were purchased as sodium salts, and indomethacin was purchased as a 99% pure powder from Sigma-Aldrich. LPS was purchased from Sigma-Aldrich, and FBS was purchased from Hyclone Laboratories. Thioglycollate media (6% thioglycollate, 1% NaCl, 14% in water) was purchased from Difco. Antimicrobial (100 units/ml penicillin, 100 μg/ml streptomycin) was purchased from Hyclone Laboratories.

**Animals**—mPGES-1−/− mice were obtained from S. Akira (Osaka University) and have been described previously (23). Sequencing of the mPGES-1 gene in cDNA isolated from the cDNA of mPGES-1 KO animals predicts a mutant mPGES-1 protein with wild type N terminus but a stop codon after amino acid 43.3 Mice (129P2/H11002, C57BL/6J, 43-64 g) were bred at Taconic Farms and housed in a 12-h light/dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care Committee and performed in strict accordance with the Animal Care and Use Committee, and the Committee for the Purpose of Control and Supervision of Experiments on Animals. Mice were injected intraperitoneally with 0.1 ml of LPS (1 μg/ml, Salmonella minnesota Re595, Sigma-Aldrich) or saline, and tissues were collected 4 h post-dosing. Tissues were flash frozen in liquid nitrogen and kept at −80°C until further analysis. All procedures were approved by the Institutional Animal Care Committee and performed in strict accordance with the Animal Care and Use Committee.

**Macrophage Preparations**—Five animals each for mPGES-1 wild type, heterozygote, and null mice were used as macrophage source and treated, as described above, to account for potential differences caused by mixed genetic background. Mice were injected intraperitoneally with 1 ml of 6% thioglycollate media. Peritoneal lavages with PBS supplemented with 10 units/ml heparin were performed 3–4 days following injection to recover thioglycollate-elicited macrophages. Resident macrophages were obtained from peritoneal lavage of naive animals. Cells were centrifuged and suspended in RPMI 1640 (Invitrogen) supplemented with 10% FBS (Invitrogen), penicillin 100 units/ml, and streptomycin 100 μg/ml (Invitrogen). Cells recovered from individual mice were plated in triplicate in 96-well dishes at a density of 2 × 10^5 cells per well and allowed to adhere for 1 h in a humidified incubator at 37°C with 5% CO_{2}. Non-adherent cells were eliminated by two PBS washes. Macrophages were incubated with culture media alone (RPMI 1640 supplemented with 2% FBS and antibiotic) or culture media containing LPS (S. minnesota Re595, Sigma-Aldrich) at 100 ng/ml for 24 h. Following the incubation, the culture media was collected and assayed for PGE_{2}, PGE_{2}a, TGFB, and 6-keto-PGF_{1α} by ELISA (Assay Design) according to the manufacturer’s instructions. Some results obtained by ELISA were subsequently confirmed by LC-MS analysis, which also allowed dosing of PGD_{2}. For Western blotting, peritoneal lavage cells were plated in 48-well dishes at a density of 5 × 10^5/well and treated as described above. Adherent macrophages were cultured with PBS and lysed with 40 μl of lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1% Nonidet P-40) supplemented with Complete™ protease inhibitor (Roche Applied Science). Half the volume of pooled samples were resolved on SDS-PAGE and processed by Western blotting.

**Tissue Processing**—Tissues were homogenized at 4°C in PBS supplemented with 1× of Complete™ protease inhibitor mixture and 20 μl indomethacin with a Polytron and sonicated for 30 s. The homogenates were first centrifuged at 9,000 × g for 10 min, and the supernatant was used for protein determination and for PG measurements. A portion of the supernatant was used to prepare microsomes by centrifugation at 100,000 × g for 90 min. The pellet was solubilized in lysis buffer (50 mM Tris-Cl, pH 7.5, 2 mM EDTA, 1% Nonidet P-40) supplemented with a protease inhibitor mixture (Complete™, Roche Applied Science). Protein content was measured by the Bradford assay (Bio-Rad) using bovine γ-globulin as standard. Fractions were kept at −80°C until further analysis.

**SDS-PAGE and Immunoblot Analysis**—Solubilized microsomes (50 μg) and macrophage extracts were resolved by electrophoresis in 10–20% SDS-Tris-glycine pre-cast gels (Invitrogen) and transferred to nitrocellulose. Quantitative Western blotting was performed with purified antibodies against COX-1, COX-2, and mPGES-1 protein standards (1, 3, and 5 ng of each) that were loaded on each gel for standardization. The following antibodies were used: anti-human mPGES-1 (Cayman #160140, 1:300), anti-COX-2 (MF243, Merck Frosst, 1:3000), anti-COX-1 (Cayman #160110, 1:500), and anti-β-actin (Sigma-A #A-5310, 1:5000) in PBS supplemented with 0.5% Tween 20 and 5% nonfat skim milk. The secondary horseradish peroxidase-linked goat anti-rabbit and anti-mouse IgG antibodies (Amersham Biosciences NA934 and NA931) were used at a dilution of 1:10,000. Immunodetection was performed with chemiluminescence using Super Signal West Femto Maximum Sensitivity substrate (Pierce). Detection and quantitative analysis were performed using a Fuji Film LAS-1000 charge-coupled device and Image Gauge software.

**Liquid Chromatography-Mass Spectrometry Prostaglandins Measurements**—PGF_{2α}, PGD_{2}, 6-keto-PGF_{1α}, and TXB_{2} were analyzed by liquid chromatography mass spectrometry (LC-MS). Samples (100 μl) were protein-precipitated by the addition of acetonitrile (150 μl) containing deuterated prostaglandins (2 ng each) as internal standard (deuterated PGE_{2} served as internal standard for both PGE_{2} and PGD_{2}). Samples were vortexed and centrifuged (4000 × g, 10 min), and the supernatant was transferred to a new 96-well plate. Samples (50 μl) were injected onto a 4.6 × 50 mm YMC ODS-A column using a Shimadzu SIL-HTc autosampler and LC-10ADVP pumps. Prostaglandins were eluted at 1 ml/min using a linear gradient from 10 to 90% acetonitrile over 30 min. Prostaglandins was achieved using a Sciex API-4000 triple quadrupole mass spectrometer. Analysis was carried out using negative ion electrospray with 1 ml/min entering the source. The source was operated at 500°C, electrospray voltage was −4500 V, and gas 1 and gas 2 were 60 and 50, respectively. Each prostaglandin was optimized individually for parent mass and fragment mass sensitivity.

**Real-Time Quantitative PCR**—Total RNA was extracted from tissues using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Briefly, tissues were homogenized in 15 volumes of TRIzol and incubated at room temperature for 10 min. Samples were then mixed with 0.2 volume of chloroform, incubated at 25°C for 5 min, and centrifuged at 13,000 × g for 10 min. The aqueous phase was added to a fresh tube containing an equal volume of isopropanol, and RNA was precipitated at 25°C for 5 min before centrifugation at 13,000 × g for 20 min (4°C). The RNA pellet was washed once with 75% (v/v) ethanol and resuspended in RNase-free water. Between 50 and 100 μg of each RNA sample were further purified on RNeasy columns (Qiagen). Genomic DNA was removed by DNeasy treatment according to the RNeasy mini kit instruction manual. Purified RNA was quantified, and RNA integrity was evaluated using an Agilent 2100 bioanalyzer apparatus. Each RNA sample was reverse-transcribed using the TaqMan reverse transcription reagent kit (Applied Biosystems), with 250 ng of total RNA per 100 μl of reverse transcription reaction mix. Each 20 μl of cDNA was used in real-time quantitative PCR, primer annealing, transcription and reaction termination were done 25°C, 48°C, and 95°C for 10 s, 30 s, and 5 min, respectively. The following primers and probes used are shown in Table S2. Each real-time quantitative PCR analysis was carried out using a 18 S rRNA primers and probe set was purchased from Applied Biosystems. All TaqMan hydrolysis probes except for the 18 S rRNA probe consisted of a gene-specific oligonucleotide dually labeled with a 6-carboxyfluorescein reporter dye at the 5'-end and a Black Hole Quencher-1 dye at the 3'-end. The 18 S rRNA probe contained a VIC reporter dye at the 5'-end and a 6-carboxytetramethylrhodamine quencher dye at the 3'-end. Primer specificity for all amplifications was confirmed by gel electrophoresis. Real-time PCR was performed on an ABI Prism 7700 sequence detection system (Applied Biosystems) in a total volume of 50 μl containing 10 μl of cDNA, 25 μl of TaqMan PCR Universal Mix, 0.8 μl of each primer, 0.4 μl of each probe, and 1 × of the TaqMan PCR Master Mix (Applied Biosystems). Analysis was performed in triplicate.
900 nM forward and reverse primers, and 300 nM TaqMan probe. PCR for the 18 S rRNA was performed in a similar way, except that 2.5 µl of the commercial primers and probe mix was used. The cycling parameters consisted of 2 min of uracil removal incubation at 50 °C, 10 min polymerase activation at 95 °C, 50 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. Every reaction set included a reaction in which water was used instead of cDNA to serve as a control for DNA contamination and probe degradation. Quantification was performed as described in User Bulletin 2 of the ABI Prism 7700 sequence detection system using the comparative Ct method.

Statistical Analysis—Standard one-way analysis of variance (ANOVA) was used to compare multiple groups. Data was log-scaled so that underlying assumptions of equal variance and normality were better satisfied. All comparisons were deemed statistically significant at the 0.05 level.

RESULTS

Effect of Deletion of mPGES-1 on PGE2 Release by Macrophages—Production of PGE2 by LPS-stimulated thioglycollate-elicited peritoneal macrophages was shown previously to depend on the expression of mPGES-1 (23). Because macrophages vary widely in their morphology and cytokine-producing properties, we asked whether mPGES-1 would play an equally essential role in a different macrophage type. To that end, we compared PGE2 accumulation in the media of thioglycollate-elicited and resident peritoneal macrophages from mPGES-1+/+ and mPGES-1−/− mice and quantified COX-2 and mPGES-1 expression in each of these cells, in the absence or presence of LPS stimulation.

As expected, LPS-stimulated mPGES-1+/+ thioglycollate-elicited peritoneal macrophages produced PGE2 in amounts that exceeded by 10-fold PGE2 released by resting cells. LPS failed to significantly increase PGE2 output by mPGES-1−/− macrophages, whereas, interestingly, the amount of PGE2 released by mPGES-1−/− cells was approximately half that of wild type cells (Fig. 1A). Unstimulated macrophages produced
low levels of PGE₂ with no clear variation attributed to genotype. A similar experiment was performed with resident peritoneal macrophages. LPS treatment resulted in a 700-fold increase in accumulated PGE₂ over a 24-h period, and per cell, resident macrophages produced ~10-fold more PGE₂ than thioglycollate-elicited macrophages. Loss of one mPGES-1 allele led to a 50% reduction in PGE₂ release, whereas complete absence reduced PGE₂ output by 96%. Despite this large reduction, LPS treatment of mPGES-1⁻/⁻ resident macrophages resulted in a 20-fold increase in accumulated PGE₂ compared with untreated mPGES-1⁻/⁻ cells (Fig. 1B). Thus, a small portion of PGE₂ was produced in the absence of mPGES-1 activity. Taken together, these results argue for an important and rate-limiting role for mPGES-1 in PGE₂ synthesis by macrophages and uncover a minor component of LPS-stimulated PGE₂ production independent of mPGES-1 in resident macrophages.

**mPGES-1 and COX-2 Expression Both Regulate Production of PGE₂**—To better understand the mPGES-1 gene dosage effect on PGE₂ release, we quantified mPGES-1 and COX-2 protein by Western blotting in resident and elicited macrophages from wild type, heterozygous, and mPGES-1-null mice. At rest, both macrophage types from wild type and heterozygous mice expressed low levels of mPGES-1 protein. LPS stimulation for 24 h increased mPGES-1 protein expression, with levels in heterozygous cells reaching only half of those found in wild type cells (Fig. 2, A and C). COX-2 protein expression was strongly induced by LPS and was not significantly different in wild type and heterozygous macrophages. Interestingly, LPS-induced COX-2 expression in resident and thioglycollate-elicited mPGES-1⁻/⁻ macrophages was 2- to 3-fold greater than the levels induced in wild type cells (p < 0.05, Fig. 2, B and D). Similar results for mPGES-1 and COX-2 were obtained with 6 h of LPS stimulation (data not shown).

The quantitation of mPGES-1 and COX-2 revealed several interesting findings. LPS-stimulated wild type resident and elicited macrophages expressed similar amounts of mPGES-1 protein per cell. However, resident macrophages released 10-times more PGE₂, which coincided with a 10-fold greater COX-2 protein content. Both macrophage types showed a 50% decrease of PGE₂ accumulation when the mPGES-1 protein content was reduced by half in heterozygous cells (Table I). These results suggest that COX-2 and mPGES-1 together regulate total PGE₂ release, with COX-2 being mainly responsible for the difference in PGE₂ output between elicited and resident cells.

**Prostanoid Synthesis in the Absence of mPGES-1**—Induction of COX-2 in the absence of mPGES-1 could lead to an increased PGH₂ availability for alternative prostanoid synthetic pathways. We examined this possibility by comparing the production of other prostanoids (the thromboxane metabolite TXB₂, PGF₂α, PGD₂, and the prostacyclin metabolite 6-keto-

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**TABLE I**

| Genotype (thioglycollate-elicited) | COX-2 | mPGES-1 | PGE₂* |
|-----------------------------------|-------|---------|-------|
| +/+                               | 8,900 | 178,000 | 2.2   |
| −/+                               | 5,500 | 65,000  | 1.1   |
| −/−                               | 17,200| 0       | 0.2   |

| Genotype (resident)               | COX-2 | mPGES-1 | PGE₂* |
|-----------------------------------|-------|---------|-------|
| +/+                               | 75,000| 173,000 | 28.3  |
| −/+                               | 49,000| 86,000  | 15.2  |
| −/−                               | 240,000| 0 | 0.7   |

* 24 h media accumulation per 200,000 plated cells.

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**Fig. 3. Prostaglandin release by macrophages lacking mPGES-1 activity.** Thioglycollate elicited (A–C) and resident (D–F) peritoneal macrophages were stimulated for 24 h with 100 ng/ml LPS. The accumulation of TXB₂, PGF₂α, and 6-keto-PGF₁α was measured in the same culture media as PGE₂ shown in Fig. 1. PGD₂ levels were below the limit of detection (40 pg/ml) of the assay. The data presented here are mean PG concentrations ± S.E. from cells derived from five different mice, plated in triplicate, for each mPGES-1 genotype. An asterisk above the bar graph indicates a statistically significant difference (p < 0.05, one-way ANOVA) with the group of interest and the mPGES-1⁻/⁻ group.

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**mPGES-1 Is Necessary for Inflammatory PGE₂ Release in Vivo**—We then determined how the absence of mPGES-1 would affect the production of prostanoids in LPS-treated mice. PGE₂ and other prostanoids were measured in tissue homogenates from groups of 6–7 mice that had been injected with either LPS or a saline solution. A significant PGE₂ elevation (p < 0.05) was observed in brain, kidney, and lung of LPS-treated wild type animals relative to saline-injected controls. In contrast, LPS resulted in a 40% decrease of gastric PGE₂ levels (p = 0.01). No PGE₂ elevation was induced by LPS in mPGES-1⁻/⁻ mice (Fig. 4, A–C). Although the average PGE₂ increases in several tissues of LPS-treated mPGES-1 heterozygous mice were lower than in wild type animals, a statistically significant difference with LPS-treated wild type mice was not reached. We conclude that mPGES-1 is necessary for LPS-inducible PGE₂ production in brain, kidney, and lung during endotoxin challenge. Interestingly, spleens and stomachs of LPS-treated mPGES-1⁻/⁻ mice contained less PGE₂ than saline-treated animals (Fig. 4, D and E), whereas stomach TXB₂ (Fig. 4F), PGF₂α, and 6-keto-PGF₁α (not shown) were significantly increased (3.5-, 2-, and 3.5-fold, respectively, p < 0.02). Kidneys...
from LPS-treated mPGES-1−/− mice exhibited a 1.3-fold (p < 0.01) elevation of PGD2 and a trend (p = 0.06) for a 1.5-fold increase of 6-keto-PGF1α content (data not shown). None of the other tissues examined (brain, heart, lung, or spleen) showed changes in prostaglandins other than PGE2 in the absence of mPGES-1. Taken together, these results show that deletion of mPGES-1 markedly reduces LPS-stimulated PGE2 production in vivo and causes tissue-specific alterations of prostanooid levels, especially in stomach and kidney.

mPGES-1 Protein Is Constitutively Expressed in Several Mouse Tissues—The expression of mPGES-1, COX-2, and COX-1 proteins in tissues of saline and LPS-treated mice was examined by Western blotting. Shown in Fig. 5 are results from 3–4 animals out of the 6–7 used in each group. Significant amounts of mPGES-1 protein were basally expressed in lung, spleen, kidney, and stomach of wild type mice but not in brain or in any tissues of mPGES-1-null mice. A detectable mPGES-1 protein elevation, above basal levels, was induced by LPS in brain and spleen of wild type mice. COX-1 protein remained unchanged, irrespectively of genotype or treatment. Basal COX-2 expression was detected in brain and lung of all saline-treated animals, without distinction with respect to genotype. LPS injection induced a variable increase of COX-2 protein expression in brain, spleen, and kidney. The COX-2 protein levels in stomach were below the limit of detection (<1 ng/40 µg microsomes; data not shown) and were significantly lower than COX-1 (>10 ng/40 µg microsomes).

mPGES-1 Contributes to Basal, Non-inflammatory PGE2 Production in Vivo—The constitutive expression of mPGES-1 protein in several tissues and sub-baseline PGE2 levels encountered in LPS-treated mPGES-1-deficient spleens and stomachs, suggested a participation of mPGES-1 to basal PGE2 synthesis. We investigated this possibility by measuring the prostanooid content in tissues of wild type, heterozygous, and mPGES-1-null mice. PGE2 was lower in mPGES-1−/− compared with wild type animals (p < 0.05) in brain (2-fold reduction), kidney (2-fold reduction), spleen (5-fold reduction), and stomach (4-fold reduction; Fig. 6, A–E). No statistically significant changes were observed in lung (Fig. 6C) and heart (data not shown). Brain, stomach, and spleen from mPGES-1-heterozygous animals showed a decrease in their mean PGE2 levels relative to wild type littermates, but the reduction was not statistically significant. Other prostaglandins were also measured. The absence of a functional mPGES-1 gene led to a significant (p < 0.05) increase in TXB2 (5.5-fold), PGF2α (2-fold), 6-keto PGF1α (1.7-fold), and PGE2 (2.2-fold) in stomach (Fig. 6, F–I). mPGES-1−/− kidneys exhibited a statistically significant increase (1.3-fold, p < 0.05) in PGD2 and non-statistically significant 1.6-fold increase in 6-keto-PGF1α (data not shown). We conclude that mPGES-1 contributes to 50% of tissue PGE2 in brain, kidney, and 80–90% in stomach and spleen, under basal conditions. The prostaglandin profile of the stomach was most affected by the lack of mPGES-1 activity.

Gastric Prostaglandin Elevation in Stomach Is Not the Result of Changes in Terminal Synthases Expression—Changes in TXB2, 6-keto-PGF1α, PGD2, and PGF2α in stomachs of mPGES-1−/− mice could result from an increase in terminal PG synthase expression or shunting of surplus PGH2 toward remaining terminal PG synthases. To distinguish between these possibilities, the expression levels of mPGES-1, mPGES-2, li-
pocalin-type PGD synthase, PGI synthase, TXA synthase, and COX-2 were measured by real-time quantitative PCR, in tissues from saline-treated wild type animals and LPS-treated wild type and mPGES-1-null animals. Primer pairs and probes used for each message are indicated in Table II. Results for kidney and stomach are shown in Fig. 7. As expected, LPS caused an elevation of the mPGES-1 and COX-2 messages in kidneys of wild type mice (Fig. 7, B and D). The variable induction of COX-2 protein measured by Western blotting correlated well with mRNA determined by quantitative PCR in individual mice (data not shown). No significant increase in the kidney expression of other terminal synthases was observed among LPS-treated wild type and mPGES-1-null animals (Fig. 7). Similar results were obtained in stomach, except that LPS treatment elicited a significant increase in TXA synthase RNA levels (Fig. 7K). Stomachs of animals in the mPGES-1−/− LPS-treated group showed a 2-fold increase in average COX-2 message level relative to wild type, however, this increase was not statistically significant (Fig. 7A). Overall, these results show that absence of mPGES-1 does not result in an elevation of the expression of terminal PG synthases. The gastric PG profile change encountered in mPGES-1 null mice may result from a combination of a minor increase in stomach COX-2 expression and PGH₂ shunting to other terminal PG synthases.

**DISCUSSION**

Using mPGES-1-deficient mice, we examined the impact of reducing or eliminating mPGES-1 activity on prostaglandin release by two macrophage types in vitro and by normal and inflamed tissues in vivo. Three major conclusions can be derived from our experiments. First, we showed that mPGES-1 is necessary in vitro for PGE₂ release by thioglycollate-elicited and resident peritoneal macrophages, in a gene-dosage-dependent manner. In parallel, we uncovered a low level of mPGES-1-independent PGE₂ releasing activity in resident peritoneal macrophages. Second, we demonstrated the necessity of mPGES-1 for PGE₂ release in several tissues of LPS-injected mice and showed for the first time a significant contribution of mPGES-1 activity to basal PGE₂ production in vivo. Finally, we found that the prostaglandin (other than PGE₂) content of most cells and tissues is not dramatically affected by the absence of mPGES-1 activity except in the stomach, where a significant increase of all other prostanoids may originate from an increase in available PGH₂ to other terminal PG synthases. We expand in greater detail on each of these findings below.

**PGE₂ Release by Macrophages**—Thioglycollate-elicited and resident peritoneal macrophages rely on mPGES-1 activity to produce PGE₂ following LPS-stimulation (Fig. 1). Interestingly, mPGES-1−/−-resident peritoneal macrophages exhibited residual PGE₂ synthesize activity when treated with LPS. Because PGH₂ can non-enzymatically degrade to PGE₂ and PGD₂ with a ratio of ~2:1 (35), it was unclear if the PGE₂ produced by resident mPGES-1−/− macrophages arose enzymatically or from the spontaneous degradation of surplus PGH₂ to PGE₂. No PGD₂ was detected in the culture media of resident mPGES-1−/− macrophages, and we therefore favor the possibility that PGE₂ was made enzymatically in these cells, possibly through the recently discovered mPGES-2.

The comparison of PGE₂ release by resident and thioglycollate-elicited peritoneal macrophages, together with quantitation of mPGES-1 and COX-2, yielded important information on the relationship of these two enzymes in cellular PEG₂ production. The larger PGE₂ synthesis capacity of resident peritoneal macrophages has been documented previously and ascribed to greater COX-2 expression and arachidonic acid-releasing activity in these cells (36, 37). Although we have not examined arachidonic acid-releasing activity, we found that a 10-fold larger COX-2 content in resident macrophages was accompa-
nied by 10-fold greater PGE2 synthesis and that a 2-fold reduction in mPGES-1 protein content in mPGES-1 \(-/-\) cells was accompanied by a 2-fold reduction in PGE2 release. Taken together, our results indicate that the rate of PGE2 synthesis in LPS-stimulated macrophages is governed by both COX-2 and mPGES-1 protein levels.

**Role of mPGES-1 in Vivo**—PGE2 synthesis in LPS-stimulated macrophages is highly dependent on mPGES-1 (23, 31). This dependence was shown in the plasma of LPS-injected mPGES-1-null mice (23). Here we demonstrated the requirement for mPGES-1 in PGE2 production in vivo, in multiple tissues of LPS-treated mice. Additionally, we discovered that mPGES-1 significantly contributes to basal PGE2 production in certain tissues, particularly the spleen (90% contribution) and the stomach (80% contribution). This finding is supported by the detection of constitutive mPGES-1 protein expression in stomach, spleen, lung, and kidney. With gastric PGE2 levels largely originating from COX-1 activity (38), our data strongly suggests the existence of a functional relationship between COX-1 and mPGES-1 that is physiologically relevant. PGE2 plays an important role in the maintenance of gastrointestinal mucosal integrity (39), and the 80% reduction of gastric PGE2 raised the possibility that mPGES-1 null mice may develop spontaneous gastric or intestinal lesions. Gross macroscopic examinations of stomachs from null mice did not reveal any abnormality.3 The 2-fold increase in COX-2 message and the significant elevation of other prostanoids may be part of a feedback mechanism in response to low PGE2 content. It will be interesting to test whether mPGES-1 null mice are more susceptible to non-steroidal anti-inflammatory drug-induced lesions. The outcome of such an experiment is difficult to predict, because, contrary to expectations, COX-1 null mice are more resistant to ASA-induced gastric erosions (38), and that the inhibition of both COX-1 and COX-2 is required to cause gastrointestinal lesions (40).

**Shunting of PGH2 to Alternative Prostanoid Pathways**—It is theoretically possible that PGH2 produced by cyclooxygenases

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3 R. Gordon, personal communication.
may be diverted to other prostanoid pathways in the absence of mPGES-1. We did not see a systematic occurrence of this phenomenon in the various cells and tissues analyzed. For example, thioglycollate-elicited macrophages showed an increased PGF$_2\alpha$-producing capacity in the absence of mPGES-1, but resident macrophages did not. More striking is the comparison of spleen and stomach of mPGES-1$^{-/-}$ mice. The stomachs of mPGES-1-null animals exhibited significant increases of all

| Gene          | Accession no. | Primers                                    | Probe                      |
|---------------|---------------|---------------------------------------------|----------------------------|
| COX-2         | M64291        | Forward: 5'-CAAGCCAGGGCCGAATCC-3'           | 5'-TTCAATCCATGTGAAAACCCTG-3' |
| mPGES-1       | AB041997      | Forward: 5'-CCGTTTCTACCCAGGAG-3'            | 5'-TGGAGCGGTGCTGAGGCAC-3'   |
| mPGES-2       | AK024100      | Forward: 5'-TGCTGCTGCGAGGTAATGC-3'          | 5'-CTTTCGAGGACTGATGCACACTC-3' |
| L-PGDS        | D83329        | Forward: 5'-AGAAGAGCTGTATGGTGACGACGAC-3'    | 5'-TAGCCCTCCTACAGGAAGCGC-3' |
| PGIS          | NM_008968     | Forward: 5'-TACACCGACCCATGAGG-3'            | 5'-CAGGCGAAGATGCTGACACTC-3' |
| TXAS          | L18868        | Forward: 5'-TCCCTCTCAGGACTGACCAAGG-3'       | 5'-CCACCCAGAGGTCCATTCACC-3' |

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**FIG. 7.** Expression of COX-2 and terminal synthase mRNAs in stomach and kidney of mPGES-1-null mice. Real-time quantitative PCR was performed on stomach and kidney samples originating from the same animals as shown in Fig. 4. Only tissues from wild type and mPGES-1-null animals were processed. Data are presented as the fold change of RNA expression relative to wild type (-LPS) control, which was arbitrarily set at 1.0. Error bars represent ± S.E. The mRNAs for the following proteins were examined: COX-2 (A and B); mPGES-1 (C and D); mPGES-2 (E and F); PGD synthase (lipocalin-type; G and H); PGI synthase (I and J); TXA synthase (K and L), in stomach (A, C, E, G, I, and K) and kidney (B, D, F, H, J, and L). White bars: wild type mice, saline injected (n = 6); gray bars: wild type mice injected with LPS (n = 7); black bars: mPGES-1 null mice injected with LPS (n = 7). Statistically significant changes (p < 0.05) between groups designated by brackets at the top of the bars is indicated by an asterisk. Primers used for real-time quantitative PCR are indicated in Table II.
prostanoids (except PGE₂), whereas no changes were noted in spleens, despite the 80% reduction in PGE₂. Deficiency of PGI synthase also resulted in an apparent increase of TXB₂ and PGE₂ in kidneys and lungs of PGIS⁺⁻/⁻ mice compared with wild type animals (41). We asked whether basal gastric PG changes in mPGES-1-null animals were due to shunting of PGG₂ to other terminal synthases, or the result of compensatory up-regulation of terminal synthase expression. Real-time quantitative PCR determination of COX-2 and other terminal PG synthases (mPGES-2, TXA synthase, PGI synthase, and lipocalin-type PGD synthase) showed no significant differences between wild type and mPGES-1-null animals. This suggests that the gastric TXB₂ increase may be due to shunting of unused PGG₂ product. Why shunting would preferentially take place in stomach and not in spleen is open to speculation. Our experiments were performed with whole stomachs, and it is still possible that terminal synthases or COX-2 may be elevated in very specific subsets of cells, and therefore contribute to the overall PG increases. A detailed immunohistochemical comparison of terminal synthases in mPGES-1 wild type and null mice would be needed to completely resolve this issue. Examination of protein expression, as opposed to mRNA induction, should be favored, especially with COX-2. LPS-induced COX-2 expression in mouse tissues shows a disproportional increase in COX-2 message compared with COX-2 protein (compare Figs. 5 and 7). The discrepancy between induction of terminal synthases in mPGES-1 wild type and null mice would be needed to completely solve this issue. Expression of terminal synthases in mPGES-1-null animals (41). We asked whether basal gastric PG changes in mPGES-1-null animals were due to shunting of PGG₂ to other terminal synthases, or the result of compensatory up-regulation of terminal synthase expression. Real-time quantitative PCR determination of COX-2 and other terminal PG synthases (mPGES-2, TXA synthase, PGI synthase, and lipocalin-type PGD synthase) showed no significant differences between wild type and mPGES-1-null animals. This suggests that the gastric TXB₂ increase may be due to shunting of unused PGG₂ product. Why shunting would preferentially take place in stomach and not in spleen is open to speculation. Our experiments were performed with whole stomachs, and it is still possible that terminal synthases or COX-2 may be elevated in very specific subsets of cells, and therefore contribute to the overall PG increases. A detailed immunohistochemical comparison of terminal synthases in mPGES-1 wild type and null mice would be needed to completely resolve this issue. Examination of protein expression, as opposed to mRNA induction, should be favored, especially with COX-2. LPS-induced COX-2 expression in mouse tissues shows a disproportional increase in COX-2 message compared with COX-2 protein (compare Figs. 5 and 7). The discrepancy between induction of terminal synthases in mPGES-1 wild type and null mice would be needed to completely solve this issue. Express...
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