Microsomal Prostaglandin E Synthase-1 Deletion Suppresses Oxidative Stress and Angiotensin II–Induced Abdominal Aortic Aneurysm Formation

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Background—Microsomal prostaglandin (PG) E_2 synthase-1 (mPGES-1) catalyzes isomerization of the cyclooxygenase product PGH_2 into PGE_2. Deletion of mPGES-1 modulates experimentally evoked pain and inflammation and retards atherogenesis. The role of mPGES-1 in abdominal aortic aneurysm is unknown.

Methods and Results—The impact of mPGES-1 deletion on formation of angiotensin II–induced abdominal aortic aneurysm was studied in mice lacking low-density lipoprotein receptor ($LDLR^{-/-}$). Male mice deficient in both mPGES-1 and LDLR (mPGES-$1^{-/-}$ $LDLR^{-/-}$) and littermate $LDLR^{-/-}$ mice were initiated on a high-fat diet at 6 months of age, followed 1 week later by continuous infusion of angiotensin II (1 μg/kg per minute) for an additional 4 weeks. Angiotensin II infusion upregulated aortic expression of cyclooxygenase-2 and mPGES-1, increased aortic macrophage recruitment and vascular nitrotyrosine staining (which reflects local oxidative stress), and augmented urinary excretion of the isoprostane 8,12-*iso*-$iPF_{2\alpha}$-VI (which reflects lipid peroxidation in vivo) and the major metabolite of PGE_2 (PGE-M). Deletion of mPGES-1 decreased both the incidence (87.5% versus 27.3%; $P=0.02$) and the severity of abdominal aortic aneurysm and depressed the aortic and systemic indices of oxidative stress. Deletion of mPGES-1 also depressed urinary PGE-M, whereas it augmented excretion of PGD_2 and PGI_2 metabolites, reflecting rediversion of the accumulated PGH_2 substrate in the double knockouts.

Conclusions—Deletion of mPGES-1 protects against abdominal aortic aneurysm formation induced by angiotensin II in hyperlipidemic mice, coincident with a reduction in oxidative stress. The potential efficacy of selective inhibition of mPGES-1 in preventing or retarding aneurysm formation warrants further investigation. **(*Circulation*. 2008;117:1302-1309.)**

Key Words: aneurysm ■ aorta ■ drugs ■ prostaglandins

Abdominal aortic aneurysm (AAA) is an inflammatory disorder characterized by localized connective tissue degradation and smooth muscle cell apoptosis, leading to aortic dilatation and rupture. AAA is estimated to cause ≈1% of all deaths among men aged 65 to 85 years in developed countries.[1] Although associated with common cardiovascular risk factors,[1,2] the pathogenesis of AAA is poorly understood. Many cases are undiagnosed until they declare clinically by aneurysm leakage or rupture, and specific treatments that retard aneurysm development or induce its regression remain to be identified. Human aortic aneurysm biopsies stain strongly for cyclooxygenase (COX)-2 ex vivo, and a preliminary study of 15 patients taking nonsteroidal antiinflammatory drugs (NSAIDs) with 63 control subjects suggested that aneurysmal growth might be retarded by such treatment.[3] Deletion or selective inhibition of COX-2, but not inhibition of COX-1, decreases AAA formation in hyperlipidemic mice.[4,5]

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Prostaglandins (PG) are formed by the action of both COX-1 and COX-2. The latter enzyme is more readily induced by inflammatory cytokines and is the dominant source of prostaglandins formed in response to lipopolysaccharide infusion in humans.[6] However, placebo-controlled trials have revealed that NSAIDs selective for inhibition of COX-2 confer a cardiovascular hazard.[7] Randomized comparisons of these drugs with traditional NSAIDs and epidemiological data are consistent with studies of human pharmacology and rodent biology in attributing this risk largely to suppression of the cardioprotective properties of COX-2–derived prostacyclin (PGI_2). This risk is likely to be modu-

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lated at the individual level by the patient's underlying cardiovascular risk, the extent of drug exposure, and concomitant therapies.[8]

Microsomal prostaglandin E synthase-1 (mPGES-1)[9,10] catalyzes the isomerization of PGH_2 into PGE_2 and is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) superfamily; it has been suggested as an alternative antiinflammatory drug target.[9] Two other PGE synthases have been identified: mPGES-2[11] and cytosolic PGES.[12,13] However, mPGES-1 is the dominant source of PGE_2 biosynthesis, at least in mice.[14] mPGES-1 is often coregulated with COX-2[15] but has been colocalized with both COX isoforms in some settings.[12,16] mPGES-1 deletion in mice has been reported to modulate experimentally evoked pain and inflammation to a degree indistinguishable from treatment with traditional NSAIDs.[17,18] It has also been implicated in immune-induced pyresis.[19] However, in contrast to the effects of inhibition or deletion of COX-2, mice deficient in mPGES-1 exhibit a favorable cardiovascular profile. Deletion of mPGES-1 does not enhance responsiveness to a thrombogenic stimulus in vivo[14] and retards atherogenesis in hyperlipidemic mice.[20] These properties may reflect rediversion of the mPGES-1 substrate, PGH_2, to PGI synthase, as biosynthesis of PGI_2 is augmented in these mice.[14,20] Such a cardiovascular profile may render selective inhibitors of mPGES-1 a more attractive option than NSAIDs selective for inhibition of COX-2 in the treatment or prevention of inflammatory disorders of the cardiovascular system, such as AAA. In this study, we report that mPGES-1 deletion has a dramatic impact on AAA formation induced by infusion of angiotensin II (Ang II) in hyperlipidemic mice lacking the low-density lipoprotein receptor ($LDLR^{-/-}$). This may reflect suppression of oxidant stress, concomitant with augmented formation of both PGI_2 and PGD_2 consequent to mPGES-1 substrate rediversion.

Methods

Knockout Mice and the Ang II Infusion Aneurysm Model

mPGES-$1^{-/-}$ $LDLR^{-/-}$ mice and their littermate controls ($LDLR^{-/-}$) were generated by intercrossing mPGES-$1^{+/-}$ $LDLR^{-/-}$ mice, as described previously.[20] Mice used in these studies had an ≈75% C57BL/6 and 25% DBA/1lacJ genetic background. The impact of mPGES-1 deletion on AAA development was studied in the Ang II–induced AAA model in $LDLR^{-/-}$ mice. Male mPGES-$1^{-/-}$ $LDLR^{-/-}$ mice and littermate $LDLR^{-/-}$ mice were initiated on a high-fat diet (0.2% cholesterol, 21% saturated fat; formula TD 88137, Harlan Teklad) at 6 months of age, followed 1 week later by continuous infusion of Ang II (Calbiochem, Darmstadt, Germany) at a rate of 1 μg/kg per minute for an additional 4 weeks. The Ang II was administered subcutaneously via Alzet osmotic minipumps (model 2004) as described previously.[21] All animals were housed according to guidelines of the Institutional Animal Care and Usage Committee of the University of Pennsylvania, and all experiments were approved by the that committee. Systolic blood pressure was measured in conscious mice throughout the time course of the study with a computerized noninvasive tail-cuff system and calculated as described previously.[14]

Preparation of Mouse Aortae and Quantification of Aneurysms

Mice were euthanized and perfused with ice-cold PBS. The abdominal aorta was dissected out, immediately put into PBS, and cleaned of adventitial fat. The maximal abdominal aortic diameter was measured with the use of a caliber under a dissection microscope, while the aorta rested in PBS buffer without any physical stretching. A >50% increase in external diameter of the abdominal aorta was used to define the occurrence of an AAA. AAA severity was classified visually as described previously[22] and also assessed by the external diameter and the wet weight of the abdominal aorta.

Analyses in Plasma

Blood was drawn from the vena cavae of CO_2-euthanized mice, and EDTA (final concentration, 10 mmol/L) was added immediately. Plasma glucose, total cholesterol, and triglyceride levels were measured enzymatically on a Cobas Fara II autoanalyzer (Roche Diagnostic Systems Inc, Nutley, NJ) with reagents from Wako Chemicals (Richmond, Va).

Real-Time Polymerase Chain Reaction Analysis of Gene Expression in Mouse Aorta

TaqMan gene expression assays (Applied Biosystems, Foster City, Calif; catalog No. 4331182) for mPGES1 (Mm00452105_m1), COX-1 (Mm00477214_m1), COX-2 (Mm00478374_m1), and Ang II receptors (AT_{1A}: Mm01957722_s1; AT_{1B}: Mm02620758_s1; AT_2: Mm01341373_m1) were performed on an ABI Prism 7900 Sequence Detection System. Results were normalized with 18S rRNA (Hs99999901_s1).

Histological Examination of Vascular Morphology

Suprarenal abdominal aortae were harvested on day 8 of the Ang II infusion. Peroxidase-quenched sections (8 μm) of OCT-embedded abdominal aorta were blocked with 3% BSA (in PBS) containing 20 μg/mL goat IgG (Jackson ImmunoResearch, West Grove, Pa) followed by incubation with primary antibodies: FITC-conjugated mouse anti-α-smooth muscle actin clone 1A4 (Sigma-Aldrich, St Louis, Mo), biotinylated rat anti-CD11b (BD Biosciences, San Jose, Calif), rabbit anti-nitrotyrosine IgG (Millipore, Billerica, Mass), rabbit anti-mouse mPGES-1 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), and rabbit anti-mouse COX-1 and COX-2 antibodies (Cayman Chemicals, Ann Arbor, Mich). Sections were then incubated with either biotinylated goat anti-rabbit IgG, mouse anti-FITC IgG (Jackson ImmunoResearch, West Grove, Pa), followed by Vectastain ABC avidin-biotin (Vector Laboratories, Burlingame, Calif) or directly with Vectastain ABC avidin-biotin, and developed with DAB (DAKO, Carpinteria, Calif). All sections were counterstained with Gill's Formulation No. 1 hematoxylin (Fisher Scientific, Fair Lawn, NJ), and isotype controls were run in parallel, with negligible staining observed in all cases. CD11b, nitrotyrosine, mPGES-1, COX-1, and COX-2 staining was performed on nonfixed OCT-embedded samples, and smooth muscle cell staining was performed on Prefer (Anatech, Battle Creek, Mich) fixed and OCT-embedded samples.

Analysis of Prostanoids

Urine was collected for 24 hours at baseline and 4 weeks after Ang II infusion. Systemic production of PGE_2, thromboxane A_2 (TxA_2), PGI_2, and PGD_2 was determined by stable isotope dilution, tandem mass spectrometric quantification of their major urinary metabolites[23]: 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGE-M), 2,3-dinor-thromboxane B_2 (Tx-M), 2,3-dinor-6-keto $PGF_{1\alpha}$ (PGI-M), and 11,15-dioxo-9α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGD-M), respectively. The nonenzymatic lipid peroxidation product, 8,12-*iso*-$iPF_{2\alpha}$-VI, was measured as previously published.[23] Metabolite levels were corrected for urinary creatinine (Oxford Biomedical Research, Oxford, Mich).

Statistical Analysis

Data are expressed as mean±SEM. Comparisons of multiple groups were performed by ANOVA and a Bonferroni post-ANOVA multiple comparison test when the ANOVA was significant. When only 2 mean values were compared, the 2-tailed Mann-Whitney *t* test was used. Differences were considered statistically significant at $P<0.05$.

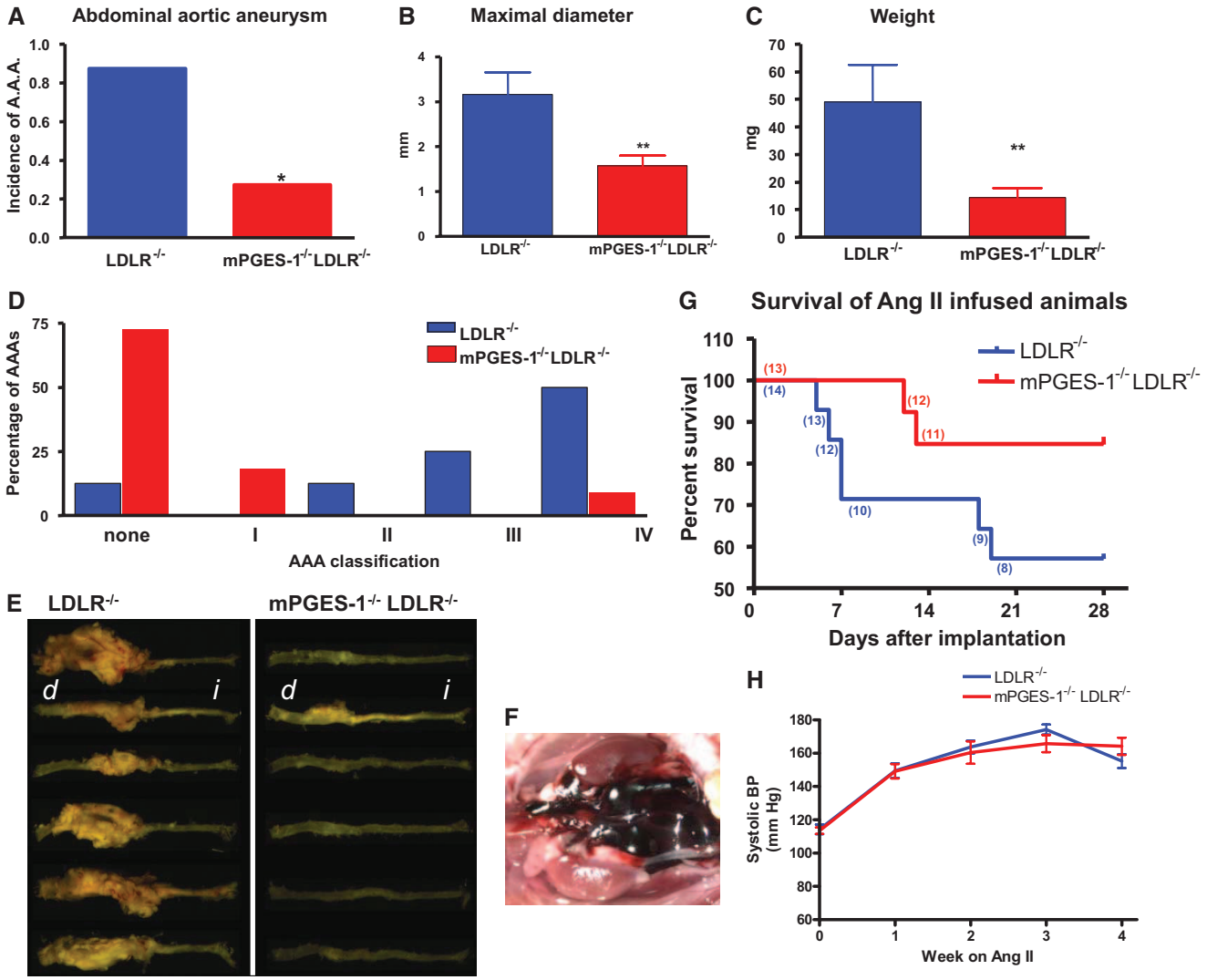

Figure 1. mPGES-1 deletion protects against aneurysm formation in LDLR$^{-/-}$ mice infused with Ang II. Deletion of mPGES-1 lowered the incidence of AAA formation (A) and decreased the severity of the disease, as reflected by maximal diameter (B) and wet weight (C) of the abdominal aorta. The AAAs were classified into 4 categories (D) according to a previous publication,[22] with category IV representing the most advanced disease. Abdominal aorta (segment starting from the diaphragm and extending to the iliac bifurcation) with representative AAAs are shown in E, with endings at diaphragm (d) and iliac bifurcation (i) indicated. Ang II infusion led to sudden death due to hemorrhage resulting from rupture of the abdominal aorta (F). The survival curve is shown in G. Blood pressure (BP) response to Ang II infusion is shown in H. A to C, n=8 and n=11 for LDLR$^{-/-}$ and mPGES-1$^{-/-}$ LDLR$^{-/-}$, respectively; *P<0.05; **P<0.01. G, Fourteen LDLR$^{-/-}$ and 13 PGES-1$^{-/-}$ LDLR$^{-/-}$ mice were studied initially, with the number of mice alive at each time interval indicated in brackets. H, Eight to 14 mice were measured at each time point.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

mPGES-1 Deletion Attenuates Ang II–Induced Aortic Aneurysm

Ang II infusion induces formation of aneurysms predominantly of the abdominal aortae in hyperlipidemic mice.[21,24] mPGES-1 deletion decreased both the incidence (LDLR$^{-/-}$: 87.5% versus mPGES-1$^{-/-}$ LDLR$^{-/-}$: 27.3%) of AAA formation and its severity, as reflected by the maximal diameter and the wet weight of the abdominal aorta (Figure 1A to 1E). Sudden death due to rupture of the abdominal aorta was both delayed and reduced in the mPGES-1$^{-/-}$ LDLR$^{-/-}$ mice (Figure 1F and 1G). Ang II infusion increased blood pressure, but a divergence was not observed between LDLR$^{-/-}$ and mPGES-1$^{-/-}$ LDLR$^{-/-}$ mice throughout the study (Figure 1H). There was no impact of mPGES-1 deletion on fasting plasma total cholesterol, free cholesterol, high-density lipoprotein cholesterol, triglycerides, or glucose in LDLR$^{-/-}$ mice (Table).

Ang II Upregulates mPGES-1 and Increases Oxidative Stress

Considering the advanced stage of most of the AAAs formed after 4 weeks of Ang II infusion (Figure 1D), we sought evidence for pathogenesis in samples harvested at an earlier time point: day 8 of Ang II treatment. The most striking morphological change at this time was that Ang II caused

Table. Plasma Biochemical Analysis in Ang II–Infused Mice

	Cholesterol, mg/dL	HDL Cholesterol, mg/dL	Triglycerides, mg/dL	Free Cholesterol, mg/dL	Cholesteryl Ester, mg/dL	Free Fatty Acid, mEq/L	Glucose, mg/dL
LDLR$^{-/-}$	317.8±4.6	37±8.2	254±45.7	117.2±9.1	200.5±12	0.478±0.036	397.3±48.7
mPGES-1$^{-/-}$ LDLR$^{-/-}$	322.4±38.2	34±3.2	275.6±78	103.8±18.1	218.6±21.2	0.624±0.081	351.2±24.7

Data are shown as mean±SEM. HDL indicates high-density lipoprotein. No statistical significance was detected between Ang II–infused mPGES-1$^{-/-}$ LDLR$^{-/-}$s and LDLR$^{-/-}$ mice. n=5 per group, except glucose determination, in which n=7 and n=10 for LDLR$^{[minus]/-}$ and mPGES-1$^{-/-}$ LDLR$^{-/-}$ mice, respectively.

macrophage recruitment to the adventitia and endothelium; however, this was not affected by deletion of mPGES-1 (Figure 2). CD11b was used in this study as a macrophage marker, although it may also identify granulocytes. The number of CD11b-positive cells (Figure 2B) was not altered by mPGES-1 deletion. Ang II increased aortic expression of both COX-2 and mPGES-1, but not COX-1, as detected by real-time polymerase chain reaction, and deletion of mPGES-1 abolished detection of its transcript, whereas expressions of COX-2 and COX-1 were not altered significantly (Figure 3A). Immunohistochemistry confirmed upregulation of mPGES-1 and COX-2 but not COX-1 in Ang II–treated LDLR$^{-/-}$ mice. mPGES-1 staining was apparent in smooth muscle cells and endothelial cells in Ang II–treated mice (Figure I in the online-only Data Supplement), whereas Ang II–induced COX-2 expression was most striking in endothelial cells in both single and double knockout mice with some staining of adventitial cells in LDLR$^{-/-}$ mice (Figure II in the online-only Data Supplement). mPGES-1 deletion significantly attenuated Ang II–induced indices of both aortic and systemic oxidative stress (Figure 3B and 3C). Peroxynitrite, the product of the reaction of O_2^- and nitric oxide, reacts with and nitrates tyrosine groups of proteins. Thus, the relative level of immunoreactive nitrotyrosine in a tissue is a widely recognized marker of general oxidative stress. Ang II treatment potently augmented immunoreactive nitrotyrosine in aortic endothelium, adventitia tunica, and tunica media (Figure 3B, top and middle panels), which is indicated by the staining of smooth muscle actin (Figure 3B, bottom panel). This effect on tissue nitrotyrosine was evident even before aneurysm formation was detected. mPGES-1 deletion significantly attenuated this index of aortic oxidative stress. The pro-oxidant effect of Ang II was also reflected by increased urinary 8,12-*iso*-iPF$_{2\alpha}$-VI, a sensitive index of lipid peroxidation in vivo.[25] Deletion of mPGES-1 depressed significantly Ang II–induced augmentation of urinary 8,12-*iso*-iPF$_{2\alpha}$-VI (Figure 3C).

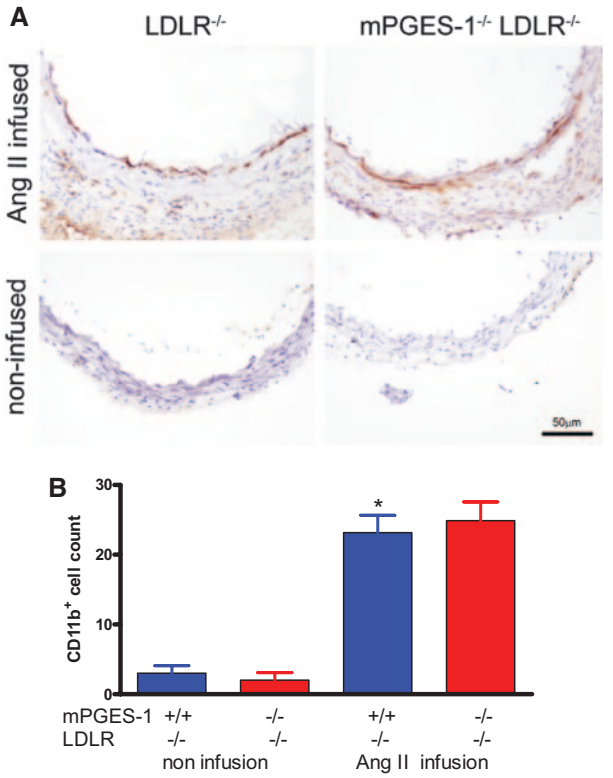

Figure 2. Aortic macrophage staining in Ang II–infused LDLR$^{-/-}$ mice. OCT sections were stained for CD11b to detect macrophages (A). Compared with control mice (bottom), Ang II infusion (top) induced recruitment of macrophage to the adventitia in both mPGES-1–competent and –deficient mice (left vs right column). Shown are representative photographs of Ang II–treated mice with 10 mice per group. Quantification of CD11b-positive cells in aortic vessel (B) wall is based on counting cells from 5 randomly selected fields of observations under ×400 magnification.

Differential Impact of mPGES-1 Deletion on Systemic Prostanoid Generation

Coincident with increased aortic expression of mPGES-1, Ang II infusion augmented systemic biosynthesis of its product, PGE_2, as reflected by urinary excretion of its major metabolite, PGE-M. This effect was abolished in mice lacking mPGES-1 (Figure 4A). Ang II increased excretion of metabolites of PGD_2, PGI_2, and TxA_2. mPGES-1 deletion augmented further the production of PGD_2 and PGI_2, but not of TxA_2, at baseline or after Ang II treatment (Figure 4B to 4D).

Ang II–induced AAA formation in mice is mediated by its receptors.[22] Deletion of mPGES-1 did not apparently affect the aortic expression of any of the angiotensin receptors: AT_{1A}, AT_{1B}, or AT_2 (Figure III in the online-only Data Supplement). An ≈60% reduction in aortic activity of matrix metalloproteinase 2 (MMP-2) was associated with mPGES-1 deficiency in the Ang II–treated mice (Figure IV in the online-only Data Supplement). Activity of aortic MMP-9 was much lower than that of MMP-2 in the same zymography study, and comparative analysis of the impact of mPGES-1 deletion on MMP-9 versus MMP-2 activity was unsuccessful.

Discussion

Studies in mice and humans have implicated COX-2 and its products in the pathogenesis of aortic aneurysm. Because this is a common but probably underdiagnosed and potentially fatal condition, the possibility of a chemopreventative strat-

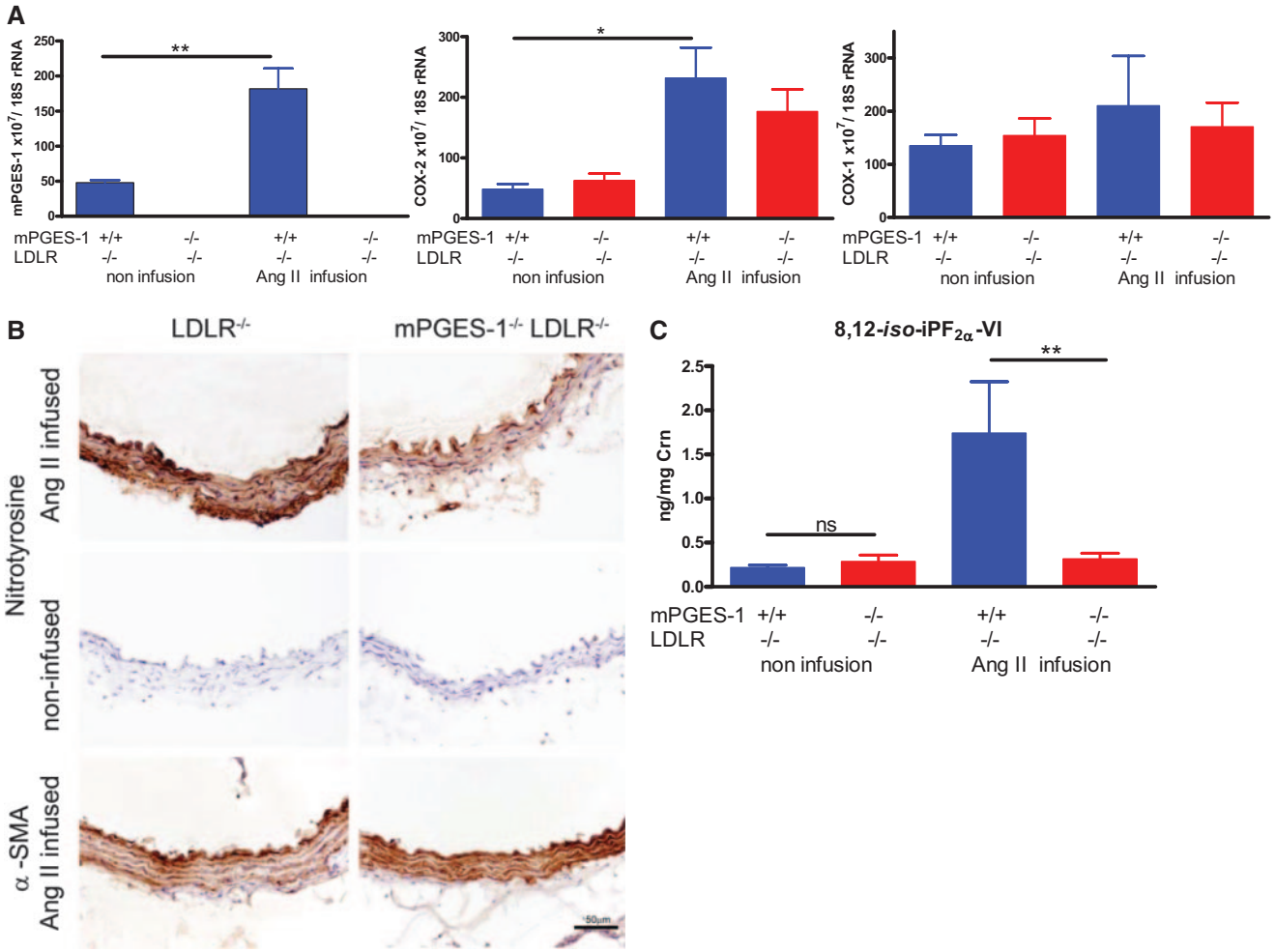

Figure 3. Upregulation of mPGES-1 coincides with increased oxidative stress during development of AAAs. Expressions of mPGES-1, COX-2, and COX-1 were examined (A, left, middle, and right panels, respectively) by real-time polymerase chain reaction in aorta from mice infused with/without Ang II. Data shown are the mean±SEM from triplicate determinations, with n=4 per group. *$P<0.05$; **$P<0.01$. Ang II infusion induced oxidative stress in abdominal aorta of both LDLR$^{-/-}$ and mPGES-1$^{-/-}$ LDLR$^{-/-}$ mice, as reflected by staining of nitrotyrosine, a marker for oxidative stress, and the induced oxidative stress was attenuated by mPGES-1 deletion (B, upper and middle panels). The induced oxidative stress appeared predominantly in the aortic media layer, as indicated by α-smooth muscle actin (α-SMA) (B, bottom panel). Shown are representative photographs of Ang II–treated mice with 10 mice per group. C, Urinary excretion of 8,12-*iso*-iPF$_{2\alpha}$-VI, a marker reflecting lipid peroxidation in vivo, was significantly depressed by deletion of mPGES-1 in mice infused with Ang II for 4 weeks. n=8 to 12 per group. Crn indicates creatinine. *$P<0.05$; **$P<0.01$.

egy has considerable appeal. However, the appreciation that NSAIDs selective for COX-2 (which include several of the older traditional NSAIDs) themselves confer a cardiovascular hazard[7] limits their utility in a population often characterized by diffuse atherosclerotic disease, a condition associated with AAA.[26] Recently, mPGES-1 has emerged as a potentially attractive alternative drug target to COX-2. Although other sources of formation of PGE_2 have been identified[11–13] and prostanoids other than PGE_2, such as PGI_2,[27,28] can mediate pain and inflammation, mPGES-1 deletion has been as effective as traditional NSAIDs in alleviating some paradigms of pain and inflammation in mice.[17,18]

In the present study, we report that mPGES-1 deletion has a marked impact on the development and extent of Ang II–induced AAA formation in mice. It is unclear to what extent this commonly used model[29] simulates the human condition. However, there is increased expression of COX-2 both in the lesions of this model and in human aneurysmal tissue,[3] and there is some preliminary evidence that prostaglandins might contribute to lesion progression in humans.[3] Patients presenting with aortic aneurysm are often characterized by extensive atherosclerotic disease. Because placebo-controlled trials[7] have shown that NSAIDs selective for inhibition of COX-2 confer a cardiovascular hazard, they are not an attractive therapeutic option despite data in mice and humans implicating COX-2 in the pathogenesis of AAA.

Initial studies in mice raise the possibility that selective inhibitors of mPGES-1 may have a more favorable cardiovascular profile than COX-2 inhibitors. We have reported previously that mPGES-1 deletion, unlike deletion or inhibition of COX-2 or deletion of the PGI_2 receptor, does not enhance the response to a thrombogenic stimulus in vivo.[14] Furthermore, unlike inhibition and deletion of COX-2, which have been reported to variably influence atherogenesis,[30] mPGES-1 deletion markedly retards lesion progression in LDLR$^{-/-}$ mice.[20] Finally, we and others have reported that

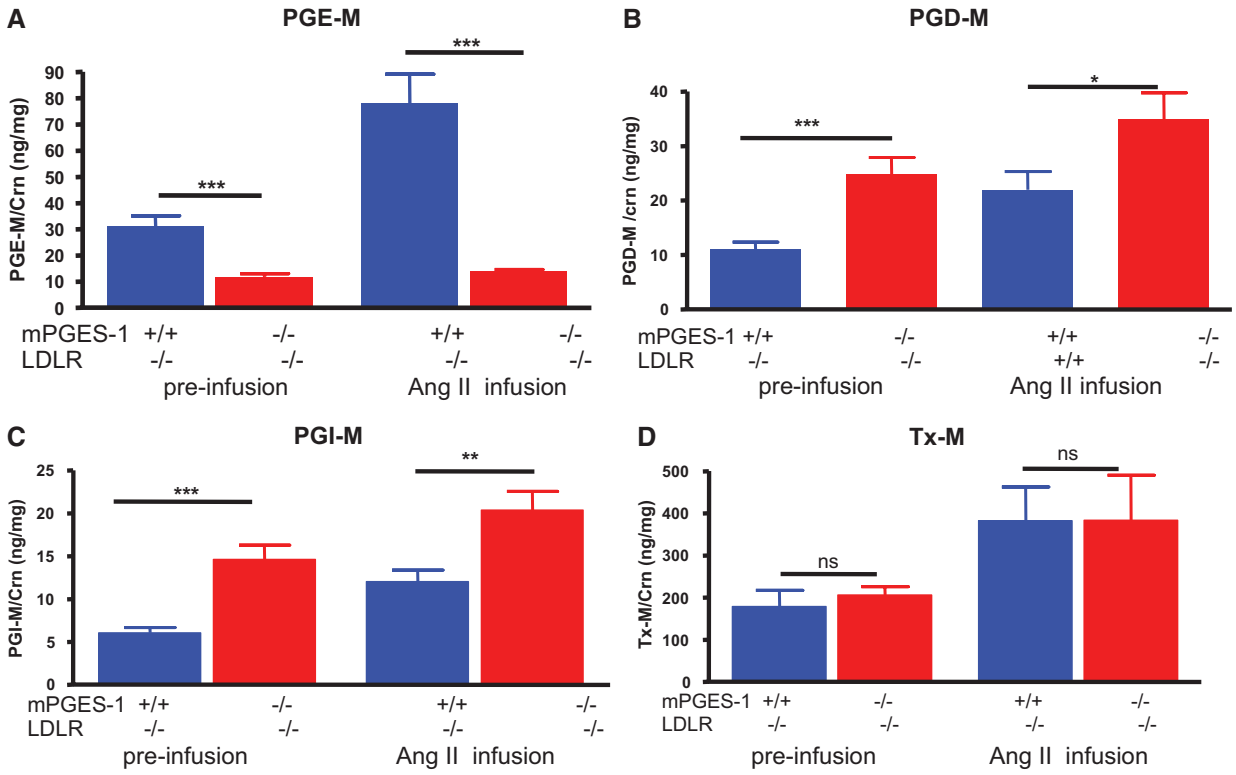

Figure 4. Biosynthesis of prostanoids in Ang II–induced formation of AAAs. Systemic production of PGE_2, PGD_2, PGI_2, and TxA_2 was examined by measuring their urinary metabolites PGE-M, PGD-M, PGI-M, and Tx-M (A, B, C, and D, respectively). Ang II infusion increased production of all these prostanoids. Deletion of mPGES-1 abolished Ang II–induced increase in PGE_2 (A). Substrate rediversion resulting from mPGES-1 deletion was seen for PGD_2 and PGI_2 but not for TxA_2 at baseline. In particular, shunting to PGD_2 and PGI_2 was sustained in Ang II–infused mice (B and C). Crn indicates creatinine. $^*P<0.05$; $^{**}P<0.01$ $^{***}P<0.001$; n=8 to 12 per group.

mPGES-1 deletion failed to elevate blood pressure, even in mice on a high-salt diet.[14,31] However, Jia et al[32] have reported a hypertensive response to mPGES-1 deletion. Potential explanations for these divergent results include differences in genetic background, a more intense salt-loading regimen, and the use of wild-type but not littermate controls. mPGES-1 deletion did not augment the hypertensive response to Ang II in hyperlipidemic mice in the present study. Although the extent to which these early observations extend to treating humans with selective mPGES-1 inhibitors remains to be established, they raise the prospect of an attractive approach to limiting inflammatory diseases in patients with established cardiovascular disease. Selective inhibitors of mPGES-1 are under development. Experiments with knockout mice suggest that they may be less likely to confer cardiovascular hazard than NSAIDs selective for inhibition of COX-2, perhaps because of substrate rediversion to cardioprotective prostanoids.

Aneurysmal lesions in both humans and mice exhibit the hallmarks of inflammation, and the attendant oxidant stress has been implicated in the pathogenesis of the disease.[33–35] In the present study, we show that infusion of Ang II results in early recruitment of macrophages and extensive evidence of oxidative stress in the vasculature, as reflected by medial staining for nitrotyrosine, especially proximal to the endothelium and the adventitia. Deletion of mPGES-1 has a marked impact on such staining as well as on a systemic index of oxidant stress[25]: urinary excretion of the most abundant F_2 isoprostane, 8,12-*iso*-$iPF_{2\alpha}$–VI. Although macrophage recruitment was unaltered by mPGES-1 deletion, the impact on oxidant stress may have reflected an alteration in their production of prostaglandins. Thus, concomitant with suppression of PGE_2, biosynthesis of both PGI_2 and PGD_2 is augmented in mice lacking mPGES-1. Both PGI_2[36] and PGD_2[37] have been implicated previously in restraining oxidant stress via their induction of antioxidant enzymes. Although Ang II increased formation of TxA_2, which has pro-oxidant properties,[38] TxA_2, unlike PGI_2 and PGD_2, was not increased further consequent to mPGES-1 deletion during AAA formation.

Prostaglandin intermediates that accumulate after inhibition or deletion of synthases downstream of the COXs may be shuttled between cells to form transcellular products of functional importance. An example is the diversion of platelet PGH_2 substrate to endothelial PGI_2 synthase,[39] which contributed to the functional effects of thromboxane synthase inhibitors.[40] Evidence for substrate rediversion within cells after mPGES-1 deletion has been shown previously.[41] The predominant products of rediversion vary according to cell type; thus, PGI_2 is by far the most abundant product in vascular smooth muscle cells, whereas both TxA_2 and PGI_2 may result from mPGES-1 deletion in macrophages.[41] Rediversion of substrate to endothelial PGI_2 might also be relevant given the upregulation of COX-2 in both endothelial cells and smooth muscle cells by Ang II infusion in the present studies. Measurement of urinary metabolites reflects alterations in

systemic biosynthesis of prostanoids and does not permit direct assignation of cellular origin to a particular product. PGD_2 is a product of macrophage COXs, and this may account for its augmented formation in the present study. However, if so, it is surprising that thromboxane biosynthesis is not altered. A more intriguing possibility is that it derives from mast cells. PGD_2 is the predominant COX product in mast cells,[42] and mast cells have previously been implicated in atherosclerotic plaque destabilization.[43] Future studies will address the origin and the functional relevance of increased formation of PGD_2 in the attenuation of AAA consequent to mPGES-1 deletion.

MMPs are thought to be involved in AAA pathogenesis.[44–46] Deletion of mPGES-1 impaired aortic MMP-2 activity (Figure IV in the online-only Data Supplement). This may be due to a direct effect of mPGES-1–derived PGE_2 and/or the suppressed vascular oxidative stress consequent to mPGES-1 deletion. Previous work has demonstrated that PGE_2 may regulate MMP-2 expression[47,48] and that suppression of oxidative stress was associated with attenuated MMP-2 activity in Ang II–infused $ApoE^{-/-}$ mice,[33] a model distinct from but similar to the one used in this study. It is presently unclear whether the reduction in activity of MMP-2 and perhaps other proteases derives from suppression of PGE_2 or substrate rediversion to other products of COX, such as PGI_2 and PGD_2.

In the present study, we provide evidence that deletion of mPGES-1 retards AAA formation and severity. This suggests the potential utility of mPGES-1 inhibitors in a condition presently not amenable to chemoprevention. Given that mPGES-1 deletion has a concomitant beneficial impact on atherogenesis,[20] mPGES-1 inhibition may have particular value in the treatment of inflammatory syndromes in patients with established cardiovascular disease.

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Disclosures

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CLINICAL PERSPECTIVE

Abdominal aortic aneurysm is an inflammatory disorder characterized by localized connective tissue degradation and smooth muscle cell apoptosis, leading to aortic dilatation and rupture. Abdominal aortic aneurysm represents a major cause of morbidity and mortality in humans. Many cases are undiagnosed until they declare clinically by leakage or rupture. Nonsurgical treatments that retard aneurysm development or induce its regression remain to be identified. Human aortic aneurysm biopsies stain strongly for cyclooxygenase-2 ex vivo, and some earlier studies provided evidence for the potential efficacy of nonsteroidal antiinflammatory drugs, particularly those selective for inhibition of cyclooxygenase-2. However, cyclooxygenase-2–selective inhibitors themselves increase the risk of myocardial infarction, heart failure, and stroke. In the present study, we explored the potential utility of targeting microsomal prostaglandin (PG) E_2 synthase-1 (mPGES-1), an antiinflammatory drug target downstream of cyclooxygenase-2 and a major source of PGE_2. We have shown previously that mPGES-1 retards atherogenesis; here gene deletion retarded formation of abdominal aortic aneurysm induced by an angiotensin II infusion in mice lacking low-density lipoprotein receptor. This occurred concomitant with suppression of aortic and systemic indices of oxidative stress, previously implicated in the pathogenesis of abdominal aortic aneurysm. Deletion of mPGES-1 inhibited production of PGE_2 but also resulted in substrate rediversion to augment production of PGI_2 and PGD_2, both of which upregulate antioxidant enzymes and restrain oxidant stress. This study raises the possibility that inhibition of mPGES-1 might have therapeutic potential in this potentially fatal disease.

Microsomal Prostaglandin E Synthase-1 Deletion Suppresses Oxidative Stress and Angiotensin II–Induced Abdominal Aortic Aneurysm Formation

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