Lack of Microsomal Prostaglandin E\textsubscript{2} Synthase-1 in Bone Marrow–Derived Myeloid Cells Impairs Left Ventricular Function and Increases Mortality After Acute Myocardial Infarction

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**Background**—Microsomal prostaglandin E\textsubscript{2} synthase-1 (mPGES-1), encoded by the \textit{Ptges} gene, catalyzes prostaglandin E\textsubscript{2} biosynthesis and is expressed by leukocytes, cardiac myocytes, and cardiac fibroblasts. \textit{Ptges}−/− mice develop more left ventricle (LV) dilation, worse LV contractile function, and higher LV end-diastolic pressure than \textit{Ptges}+/+ mice after myocardial infarction. In this study, we define the role of mPGES-1 in bone marrow–derived leukocytes in the recovery of LV function after coronary ligation.

**Methods and Results**—Cardiac structure and function in \textit{Ptges}+/+ mice with \textit{Ptges}+/+ bone marrow (BM+/+) and \textit{Ptges}+/− mice with \textit{Ptges}+/− BM (BM−/−) were assessed by morphometric analysis, echocardiography, and invasive hemodynamics before and 7 and 28 days after myocardial infarction. Prostaglandin levels and prostaglandin biosynthetic enzyme gene expression were measured by liquid chromatography–tandem mass spectrometry and real-time polymerase chain reaction, immunoblotting, immunohistochemistry, and immunofluorescence microscopy, respectively. After myocardial infarction, BM−/− mice had more LV dilation, worse LV systolic and diastolic function, higher LV end-diastolic pressure, more cardiomyocyte hypertrophy, and higher mortality but similar infarct size and pulmonary edema compared with BM+/+ mice. BM−/− mice also had higher levels of COX-1 protein and more leukocytes in the infarct, but not the viable LV, than BM+/+ mice. Levels of prostaglandin E\textsubscript{2} were higher in the infarct and viable myocardium of BM−/− mice than in BM+/+ mice.

**Conclusions**—Lack of mPGES-1 in bone marrow–derived leukocytes negatively regulates COX-1 expression, prostaglandin E\textsubscript{2} biosynthesis, and inflammation in the infarct and leads to impaired LV function, adverse LV remodeling, and decreased survival after acute myocardial infarction. *(Circulation. 2012;125:2904-2913.)*

**Key Words:** leukocytes ■ myocardial infarction ■ prostaglandins ■ remodeling ■ chimeric mice

Ischemic heart disease and myocardial infarction (MI) will be the leading cause of death worldwide by 2020.¹ MI leads to an inflammatory response characterized by the generation of proinflammatory mediators and the influx of leukocytes that are necessary to remove necrotic cellular debris and promote recovery of left ventricular (LV) contractile function. An improperly regulated inflammatory response and pathological LV remodeling can impair LV function and lead to heart failure and death after MI.² ³

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Prostaglandins, synthesized by the sequential action of phospholipase A\textsubscript{2} (PLA\textsubscript{2}), cyclooxygenases (COX-1 and/or

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COX-2), and terminal prostaglandin synthase enzymes, participate in the regulation of the inflammatory response after MI. Prostaglandin E2 (PGE2) is the principal prostaglandin generated by cardiac myocytes. The terminal step in PGE2 biosynthesis may be catalyzed by the constitutively expressed enzymes cytosolic or microsomal PGE2 synthase-2 (cPGES and mPGES-2, respectively), or by mPGES-1, an inducible enzyme. mPGES-1 is encoded by the Ptges gene and can be expressed by leukocytes, cardiac fibroblasts, and cardiac myocytes.

Inhibition of the PGE2 receptor EP4 attenuates cardiomyocyte hypertrophy in vitro, and deletion of EP4 exacerbates myocardial ischemia/reperfusion injury in vivo. In addition, Ptges mice develop more LV dilation, worse LV contractile function, and higher LV end-diastolic pressure than Ptges mice after MI. Collectively, these observations suggest a beneficial role for mPGES-1-mediated PGE2 biosynthesis in postinfarction LV remodeling. The cellular source of mPGES-1 in the heart after MI has not been identified. In this study, we show that deletion of mPGES-1 in bone marrow–derived leukocytes results in a more intense inflammatory response, pathological LV remodeling, and increased mortality after acute MI in vivo.

Methods
Reagents were from Sigma Chemical Co (St. Louis, MO) unless otherwise stated. Construction of the mPGES-1–deficient mouse line (Ptges, DBA/1acJ background), real-time quantitative polymerase chain reaction studies, 2-dimensional echocardiography, invasive hemodynamic assessment of LV function, morphometric assessment of hearts that were perfusion fixed at an intraventricular pressure of 20 mm Hg in situ, liquid chromatography–tandem mass spectrometry–based evaluation of prostanoid levels in cardiac tissue, immunoblotting, and lectin and Picrosirius Red staining were performed exactly as described previously. Housing and experimental procedures were approved by the Animal Care Committee of the University Health Network and were in accordance with the Guide for the Care and Use of Laboratory Animals research statutes, Ontario (1980).

MI Model
Mice were sedated with ketamine (50 mg/kg) and xylazine (5 mg/kg), intubated, ventilated, and maintained with 2% isoflurane. Through a left thoracotomy, the left coronary artery was ligated 1 to 2 mm below the border of the left atrial appendage. Ischemia was confirmed by the appearance of hypokinesis and pallor distal to the occlusion and by ST elevation on ECG.

Multiple Epitope Ligand Cartography
Multiple epitope ligand cartography was performed as described previously. Briefly, a slide with a cardiac section was placed on the stage of an inverted wide-field fluorescence microscope (Leica DM ILRE2). By an automated process, tissue slices were incubated for 15 minutes with a fluorescence-labeled antibody and rinsed with wash solution, and fluorescence signals were imaged. A bleaching step was then performed to delete the fluorescence signal before addition of the next antibody. Postbleaching images were recorded and subtracted from the following fluorescence image.

Bone marrow transplantation and the generation of chimeric mice, isolation and coculture of cardiac fibroblasts and bone marrow–derived mononuclear cells/macrophages, antisera for immunohistochemistry, primer sequences for real-time reverse-transcription polymerase chain reaction analysis, and the statistical analysis appear in the online-only Data Supplement.

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
LV Dilation, Impaired LV Systolic and Diastolic Function, and Increased Mortality After MI in Mice That Lack mPGES-1 in Bone Marrow–Derived Cells
To define the role of mPGES-1 in myeloid cells in the pathophysiology of acute MI, we generated chimeric mice after MI. Collectively, these observations suggest a beneficial role for mPGES-1-mediated PGE2 biosynthesis in postinfarction LV remodeling. The decrease in LV fractional shortening in BM mice 7 days after coronary ligation was comparable to that in BM mice 28 days after MI. Collectively, these findings established accelerated deterioration of cardiac function in BM compared with BM chimeras after MI. Although stroke volume and cardiac output in BM mice recovered by 28 days after MI to baseline levels, possibly reflecting compensatory remodeling (Table), fractional shortening, stroke volume, and cardiac output remained below baseline levels in BM mice (Figure 1F: Table) 28 days after MI.

The surface area of cardiac myocytes, a measure of cardiomyocyte hypertrophy, increased more in BM than BM mice 28 days after MI (Figures 1L and 1M). Despite differences in cardiomyocyte hypertrophy and cardiac function, respiratory rate (Table) and the pulmonary wet-to-dry weight ratio (Figure 1K) were similar in BM and BM mice after MI. In addition, there was no difference in collagen remodeling (Figures 1N and 1O), matrix metalloproteinase-2 or -9 mRNA expression (online-only Data Supplement Figure I), endothelial cell growth (CD31; online-only Data Supple-
Figure 1. Lack of microsomal prostaglandin E2 synthase-1 (mPGES-1) in myeloid cells leads to adverse left ventricular (LV) remodeling and decreased survival after myocardial infarction (MI). A, Ratio of SRY/GAPDH DNA after MI in bone marrow (BM) cells after irradiation of female Ptges+/+ mice and transplantation with BM from male Ptges+/+ (BM+/+; open bars) or Ptges−/− mice (BM−/−; solid bars). B, Survival of BM+/+ and BM−/− mice after MI (P<0.04, log-rank test). C, Masson’s trichrome staining of explanted hearts after hyperkalemic arrest and perfusion fixation in situ at physiological pressure. D, LV volume; E, volume of infarcted myocardium; F, fractional shortening; G, +dP/dt; H, −dP/dt; I, τ; and J, LV end-diastolic pressure. Invasive hemodynamic assessment of LV function at baseline (t=0) was not possible, because the catheter was too large to fit in the LV before infarction. K, Pulmonary wet to dry weight ratio. L and M, Lectin stain (L) and cardiomyocyte surface area (M). N and O, Picrosirius Red stain (N) and collagen content (O). Open circles or bars indicate BM+/+ mice; solid circles or bars, BM−/− mice. a, P<0.05 baseline vs 7 or 28 days after MI; b, P<0.05, BM+/+ vs BM−/− mice at any time point. Data represent 9 independent experiments for each group.

Taken together, these results provide direct evidence that loss of mPGES-1 in bone marrow–derived myeloid cells leads to impaired LV systolic and diastolic function and is associated with increased mortality after coronary occlusion. Compared with BM+/+ mice, BM−/− mice that survived 28 days after MI had impending LV failure, manifested as LV dilation and an increase in LV end-diastolic pressure, but did not develop overt pulmonary edema. Because a lack of mPGES-1 in bone marrow–derived myeloid cells has no effect on infarct volume after coronary ligation but increases LV volume, LV end-diastolic pressure, and cardiomyocyte hypertrophy, these data are consistent with the notion that lack of mPGES-1 in bone marrow–derived myeloid cells impairs LV remodeling 28 days after MI in these mice.
Lack of mPGES-1 in Bone Marrow–Derived Cells Increases the Inflammatory Response to MI

MI stimulates an inflammatory response that is characterized by an increase in cytokine and chemokine gene expression and leukocyte recruitment to the heart. An appropriately regulated inflammatory response is necessary for physiological LV remodeling and healing after MI.\textsuperscript{16,17} To assess the role of mPGES-1 in bone marrow–derived cells in the inflammatory response to MI, we studied the expression of a panel of genes that regulate inflammation in BM\textsuperscript{+/+} and BM\textsuperscript{−/−} mice. Levels of interleukin-1β (IL-1β), IL-1β receptor antagonist, and tumor necrosis factor-α mRNA in the infarct region increased significantly after MI in both chimera, and the expression of these genes was higher in BM\textsuperscript{−/−} than BM\textsuperscript{+/+} mice 7 days after MI (Figures 2A through 2C). Levels of monocyte chemotactic protein-1 and macrophage inflammatory protein-2 mRNA in the infarct also increased after MI in these mice (Figure 2F). There was no difference in the expression of IL-1\textbeta after MI in these mice (Figure 2F). There was no difference in the expression of IL-1\textbeta, IL-1\textbeta receptor antagonist, tumor necrosis factor-α, monocyte chemotactic protein-1, macrophage inflammatory protein-2, or keratinocyte-derived chemokine in the LV remote from the infarct between BM\textsuperscript{−/−} and BM\textsuperscript{+/+} mice (online-only Data Supplement Figure IV)

Next, we measured tissue levels of myeloperoxidase (MPO), an enzyme contained in the granules of neutrophils and monocytes that is released on leukocyte activation and that has been used as a marker of leukocyte infiltration into the heart after MI.\textsuperscript{16,17} Levels of MPO were significantly higher in the infarct of BM\textsuperscript{−/−} than BM\textsuperscript{+/+} mice 7 but not 28 days after MI (Figures 2G and 2H). Conversely, there was no difference in MPO protein levels in the LV remote from the infarct between BM\textsuperscript{−/−} and BM\textsuperscript{+/+} mice after coronary ligation (online-only Data Supplement Figure IV).

To independently confirm the observation that leukocyte infiltration was augmented in the infarct of mice that lacked mPGES-1 in bone marrow–derived cells, we performed immunohistochemical analysis of CD45, which is expressed on leukocytes, and MPO before and 7 and 28 days after MI. Consistent with the results of the MPO immunoblotting studies, we found that levels of both CD45 (Figures 2I and 2J) and MPO (online-only Data Supplement Figure V) were significantly higher in the infarct of BM\textsuperscript{−/−} than BM\textsuperscript{+/+} mice 7 but not 28 days after MI. Taken together, these results demonstrate that lack of mPGES-1 in bone marrow–derived cells increases the inflammatory response to MI.

Increased LV COX-1 Expression and Prostaglandin Levels in BM\textsuperscript{−/−} Mice After MI

Targeted deletion of mPGES-1 leads to alterations in the biosynthesis of multiple prostaglandins in macrophages,\textsuperscript{7} and macrophages are the most prominent inflammatory cells in the infarct zone 7 days after MI.\textsuperscript{18} To explore whether changes in prostaglandin concentrations may explain the differences in leukocyte recruitment between BM\textsuperscript{−/−} and BM\textsuperscript{+/+} mice after coronary ligation, we measured levels of prostaglandins in the infarct zone and in the viable portion of the LV adjacent to the infarct by liquid chromatography–tandem mass spectrometry. Concentrations of PGE\textsubscript{2}, thromboxane B\textsubscript{2} (TXB\textsubscript{2}, a stable Tx\textsubscript{A}\textsubscript{2} metabolite), PGF\textsubscript{2α}, 6 keto-PGF\textsubscript{1α} (a stable PGI\textsubscript{2} metabolite), and total prostaglandins were significantly higher in the infarct of BM\textsuperscript{−/−} than

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**Table. Cardiac Dimensions and Function Before and 7 and 28 Days After Left Coronary Artery Ligation**

<table>
<thead>
<tr>
<th>Days After Coronary Ligation</th>
<th>0 (n=14)</th>
<th>7 (n=13)</th>
<th>28 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BM\textsuperscript{−/−}</strong></td>
<td><strong>BM\textsuperscript{−/−}</strong></td>
<td><strong>BM\textsuperscript{−/−}</strong></td>
<td><strong>BM\textsuperscript{−/−}</strong></td>
</tr>
<tr>
<td>LV diameter, end systole, mm§</td>
<td>2.5±0.05</td>
<td>2.6±0.07</td>
<td>3.2±0.06*</td>
</tr>
<tr>
<td>LV diameter, end diastole, mm§</td>
<td>3.6±0.04</td>
<td>3.7±0.06</td>
<td>3.9±0.06*</td>
</tr>
<tr>
<td>LV volume, end systole, mL‡</td>
<td>22.1±1.1</td>
<td>26.2±2.0</td>
<td>40.1±1.9*</td>
</tr>
<tr>
<td>LV volume, end diastole, mL‡</td>
<td>54.1±6.6</td>
<td>58.2±7.6</td>
<td>66.2±7.6</td>
</tr>
<tr>
<td>Stroke volume, mL‡</td>
<td>31.9±0.9</td>
<td>33.1±1.8</td>
<td>26.1±1.4</td>
</tr>
<tr>
<td>Cardiac output, mL/min‡</td>
<td>15±0.6</td>
<td>16±0.8</td>
<td>13±0.5*</td>
</tr>
<tr>
<td>Heart rate, bpm‡</td>
<td>491±9</td>
<td>491±15</td>
<td>490±11</td>
</tr>
<tr>
<td>LV volume, mL§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LV diameter, mm§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Interventricular septum, mm§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>ND</td>
<td>ND</td>
<td>163±11</td>
</tr>
</tbody>
</table>

BM indicates bone marrow; LV, left ventricular; and ND, not determined.

*P<0.05, 0 vs 7 or 28 d post-MI.
†P<0.05, BM\textsuperscript{−/−} vs BM\textsuperscript{+/+} mice.
§In vivo 2-dimensional echocardiography.

Two-way ANOVA, followed by paired t tests, 2-tailed, unequal variance. Data represent ≥9 independent experiments for each group.
Similarly, PGE₂, PGD₂, TxB₂, 6 keto-PGF₁α increased prostaglandin levels in the infarct and LV of Figure VI, A through F). 28 days after coronary ligation (online-only Data Supplement BM than BM compared with BM mice 7 but not 28 days after coronary ligation (online-only Data Supplement Figure VI). mRNA levels of COX-1, a constitutive enzyme, and COX-2, an inducible enzyme, were higher in the infarct zone of BM/−/− mice 7 and 28 days after MI (Figures 3G and 3H). The level of mPGES-1 mRNA in the infarct was also higher in BM/−/− mice 7 and 28 days after MI (Figure 3I). The source of the increase in mPGES-1 mRNA in the infarct of BM/−/− mice must have been resident cardiac cells, because bone marrow–derived cells recruited to the heart in these mice were from Ptges−/− mice (Figure 1A), which do not express mPGES-1 mRNA. There was no change in mPGES-2 mRNA levels in either chimera 7 or 28 days after coronary ligation (data not shown).

Next, we performed immunohistochemical analysis of cardiac sections to localize COX-1, COX-2, and mPGES-1 proteins after MI (Figure 3; online-only Data Supplement). COX-1 protein levels in the infarct were higher in both BM/−/− mice 7 but not 28 days after MI (Figure 3I). Conversely, the level of COX-2 protein in the infarct was higher in BM/−/− mice 7 but not 28 days after MI (Figure 3I). The lack of microsomal prostaglandin E synthase-1 (mPGES-1) in bone marrow (BM)–derived cells increases the inflammatory response to myocardial infarction (MI). Ratio of interleukin-1β (IL-1β; A), IL-1β receptor antagonist (B), tumor necrosis factor-α (TNF-α; C), monocyte chemotactic protein-1 (MCP-1; D), macrophage inflammatory protein-2 (MIP-2; E), and keratinocyte-derived chemokine (KC; F) to GAPDH mRNA before and 7 and 28 days after MI in the infarct. G, Representative immunoblot analysis of myeloperoxidase (MPO) and GAPDH protein from 4 BM/−/− and BM/+/+ mice. H, Densitometric analysis of the ratio of MPO to GAPDH protein. I, Immunohistochemical analysis of CD45 before and 7 and 28 days after MI (magnification ×200). J, Percentage of CD45-positive cells per high-power field (indicated by arrow) before and 7 and 28 days after MI. Open bars indicate BM/−/− mice; solid bars, BM/+/+ mice. a, P<0.05 baseline vs 7 or 28 days after MI, BM/−/− or BM/+/+ mice; b, P<0.05, BM/−/− vs BM/+/+ mice at any time point. Scale bars indicate 100 μm. Data represent ≥6 independent experiments for each group.
Cardiac Fibroblasts Express COX-1, COX-2, and mPGES-1 Protein in Infarcted Myocardium

To identify the resident cardiac cells that expressed mPGES-1 (Figure 4B) and COX-1 and COX-2 protein in the infarct of these chimeras, we performed multiple epitope ligand cartography–based evaluation of the LV of Ptges+/+ and Ptges−/− mice and in BM+/+ and BM−/− chimeras 7 days after MI (the time when differences in prostaglandin levels between BM+/+ and BM−/− chimeras were noted; Figures 3A through 3F and online-only Data Supplement Figure VI, A through F). Multiple epitope ligand cartography enables sequential immunofluorescence-based visualization of multiple proteins in a tissue sample and can be used to identify the cell type that an individual protein is expressed in, and to determine which proteins colocalize in individual cells.19

In Ptges+/+ mice, mPGES-1 localized with COX-1 and COX-2 in cardiac fibroblasts but was not expressed in cardiomyocytes after MI (online-only Data Supplement Figure VIII). As expected, there was no mPGES-1 staining in the LV of Ptges−/− mice, which served as a negative control, after MI (online-only Data Supplement Figure IX). In BM+/+ chimeras, mPGES-1 colocalized with COX-1 and COX-2 in cardiac fibroblasts (Figures 4C through 4F; online-only Data Supplement Figure X). Because these cells also express PLA2 enzymes,20 cardiac fibroblasts have all of the enzymatic machinery necessary to catalyze PGE2 biosynthesis. In con-
contrast, cardiomyocytes did not express mPGES-1 in BM\textsuperscript{+/+} mice (Figure 4G) after MI. In BM\textsuperscript{−−} chimeras, mPGES-1 also colocalized with COX-1 and COX-2 in cardiac fibroblasts but did not colocalize with cardiomyocytes (Figures 4C through 4G; online-only Data Supplement Figure X). Taken together, these findings support the conclusion that the cells that stained positively for mPGES-1 on immunohistochemical analysis of BM\textsuperscript{−−} mice 7 days after MI (Figure 4B) were most likely cardiac fibroblasts.

**Higher PGE\textsubscript{2} Production by Cardiac Fibroblasts Than Mononuclear Cells/Macrophages In Vitro**

The relative capacity of cardiac fibroblasts and macrophages, the most prevalent inflammatory cell recruited to the infarct zone 7 days after MI,\textsuperscript{18} to generate PGE\textsubscript{2} in vitro was evaluated next. Treatment with IL-1\textbeta increased PGE\textsubscript{2} production in vitro by bone marrow–derived mononuclear cells/macrophages and by cardiac fibroblasts from Ptges\textsuperscript{+/+} mice (Figure 5A). On a per cell basis, cardiac fibroblasts produced 8.8-fold more PGE\textsubscript{2} than bone marrow–derived mononuclear cells/macrophages after exposure to IL-1\textbeta in vitro (Figure 5A). Although the protocol used generated a cellular population that was highly enriched in cardiac fibroblasts (online-only Data Supplement Methods), it is possible that other cells in this preparation also produced PGE\textsubscript{2} in response to IL-1\textbeta.

Because PGH\textsubscript{2} generated by macrophages recruited to the infarct may diffuse out of these cells and be metabolized to PGE\textsubscript{2} by mPGES-1 in adjacent cardiac fibroblasts, we evaluated the effect of coculturing cardiac fibroblasts from Ptges\textsuperscript{+/+} mice with mononuclear cells/macrophages from Ptges\textsuperscript{+/+} or Ptges\textsuperscript{−−} mice in vitro. There is no difference in the level of PGE\textsubscript{2} in the supernatant of Ptges\textsuperscript{+/+} cardiac fibroblasts after coculture with mononuclear cells/macrophages from either Ptges\textsuperscript{+/+} or Ptges\textsuperscript{−−} mice and treatment with vehicle or IL-1\textbeta in vitro (Figure 5B).

**Discussion**

To the best of our knowledge, this is the first study to demonstrate that a prostaglandin biosynthetic enzyme in bone marrow–derived leukocytes, in this case mPGES-1, can regulate leukocyte infiltration, cardiomyocyte hypertrophy, LV systolic and diastolic function, and survival after MI. These findings are physiologically important because they suggest that mPGES-1 in bone marrow–derived leukocytes prevents pathological LV remodeling in a clinically relevant model of coronary artery occlusion in vivo.
The molecular mechanism that leads to pathological LV remodeling in \(BM^{−/−}\) compared with \(BM^{+/+}\) mice may be related to the observation that levels of PGE\(_2\) and other prostaglandins are higher in the viable LV adjacent to the infarct in \(BM^{−/−}\) mice than in \(BM^{+/+}\) mice. Importantly, there was no difference in the expression of prostaglandin biosynthetic enzymes in the viable LV adjacent to the infarct between these chimeras, so the differences in prostaglandin levels in the viable LV adjacent to the infarct cannot be explained by local differences in prostaglandin production. On the basis of these observations, we propose a novel model in which prostaglandins or their precursor PGH\(_2\) generated in the infarct, diffuse into the surrounding viable LV, interact with prostaglandin receptors on cardiomyocytes, and act as paracrine regulators of cardiomyocyte hypertrophy and LV remodeling in vivo.

The present in vivo data do not permit identification of the prostaglandin(s) that modulate cardiac myocyte hypertrophy and pathological LV remodeling in \(BM^{−/−}\) mice after MI. That limitation notwithstanding, the independent observations that (1) PGE\(_2\) stimulates cardiac myocyte hypertrophy in vitro,\(^4,10,22\) (2) inhibition or deletion of the PGE\(_2\) receptor EP4 attenuates cardiomyocyte hypertrophy in vitro\(^10\) and in vivo,\(^21\) respectively, (3) \(Ptges^{−/−}\) mice have lower levels of PGE\(_2\) and less cardiac myocyte hypertrophy than \(Ptges^{+/+}\) mice after MI,\(^12\) (4) \(Ptges^{−/−}\) mice have an impaired cardiac hypertrophic response compared with \(Ptges^{+/+}\) mice to angiotensin II infusion in vivo,\(^23\) and (5) \(BM^{−/−}\) mice have higher levels of PGE\(_2\) and more cardiac myocyte hypertrophy than \(BM^{+/+}\) mice (cf Figures 1 and 3) strongly suggest that PGE\(_2\) directly regulates cardiac myocyte hypertrophy after MI in vivo.

Independent molecular mechanisms may account for the increased levels of PGE\(_2\) and other prostaglandins in the LV of \(BM^{−/−}\) mice 7 days after MI. For example, COX-1 catalyzes the biosynthesis of PGH\(_2\), the precursor of all prostaglandins, and COX-1 mRNA and protein expression is higher in the infarct of \(BM^{−/−}\) than \(BM^{+/+}\) mice. Although inflammatory responses are commonly associated with increased COX-2 expression,\(^24\) induction of COX-1 has been observed during inflammatory responses and cellular differentiation.\(^25,26\) It is possible that IL-1\(β\) stimulates COX-1 expression in \(BM^{−/−}\) mice after MI, because IL-1\(β\) can stimulate COX-1 expression in cultured fibroblasts under certain conditions,\(^27\) and coronary ligation leads to higher IL-1\(β\) mRNA levels in the infarct of \(BM^{−/−}\) than \(BM^{+/+}\) mice (Figure 2A). In addition, targeted deletion of COX-2 can lead to a compensatory increase in COX-1 expression in some biological systems,\(^28,29\) so it is possible that lack of COX-2–mPGES-1–catalyzed PGE\(_2\) biosynthesis leads to a compensatory increase in COX-1 expression in \(BM^{−/−}\) mice.

Because mPGES-2 and cPGES can also catalyze the terminal step in PGE\(_2\) biosynthesis, the increase in PGE\(_2\) levels in the heart of \(BM^{−/−}\) mice could be catalyzed by COX-1–mPGES-2– or COX-1–cPGES–mediated pathways. Alternatively, cardiac fibroblasts, which express COX-1, COX-2, and mPGES-1 (Figure 4) and make \(8.8\)-fold more PGE\(_2\) on a per cell basis than mononuclear cells/macrophages in vitro after treatment with IL-1\(β\) (cf Figure 5A), may be a direct source of PGE\(_2\) production in the heart after MI. A third possibility is that in inflammatory leukocytes recruited to the heart of \(BM^{−/−}\) chimeras after MI, PGH\(_2\) catalyzed by COX-1 or COX-2 builds up in these cells, because it is not metabolized by mPGES-1. This excess PGH\(_2\) may diffuse out of inflammatory leukocytes and be metabolized to PGE\(_2\) by mPGES-1 in adjacent cardiac fibroblasts. Although transcellular prostaglandin metabolism has been observed in other biological systems\(^30\) and could explain the increase in PGE\(_2\) levels in the infarct of \(BM^{−/−}\) compared with \(BM^{+/+}\) mice after MI, our failure to observe differences in PGE\(_2\) production when cardiac fibroblasts from \(Ptges^{+/+}\) mice were cocultured with mononuclear cells/macrophages from \(Ptges^{−/−}\) or \(Ptges^{+/+}\) mice mitigates against this hypothesis. A limitation of the present study is that our in vivo and in vitro data do not permit us to clearly identify which specific molecular pathway(s) or cells regulate PGE\(_2\) production in the LV after MI.

MI stimulates an inflammatory response that is characterized by the sequential recruitment of polymorphonuclear leukocytes, macrophages, and monocytes that are necessary to clear necrotic debris and to promote matrix deposition, granulatation tissue formation, angiogenesis, and infarct healing.\(^18\) In addition, leukocyte-mediated oxidation reactions play a critical role in LV remodeling after MI.\(^16\) Perturbations
in the inflammatory response are known to impair the recovery of LV function after infarction.\textsuperscript{31,32} The observation that lack of mPGES-1 in bone marrow-derived cells increases IL-1β, IL-1β receptor antagonist, and tumor necrosis factor-a mRNA expression and leukocyte recruitment to the infarct (cf Figure 2) may explain the adverse LV remodeling that we observed in $BM^{-/-}$ compared with $BM^{+/+}$ mice after MI.

Inhibition of COX-2 leads to an imbalance of prothrombotic prostaglandins (increased TxA\textsubscript{2}) and anti-thrombotic prostaglandins (decreased PGI\textsubscript{2}) that is proposed to increase the risk of MI and stroke\textsuperscript{13} and to increase mortality after MI.\textsuperscript{34} Deletion of mPGES-1, downstream from COX-2 in the inducible PGE\textsubscript{2} biosynthetic cascade, decreases brain ischemia-reperfusion injury,\textsuperscript{35} plaque burden in fat-fed Ptges\textsubscript{−/−} low-density lipoprotein receptor–deficient (LDLR\textsuperscript{−/−}) mice,\textsuperscript{36} aortic aneurysm formation,\textsuperscript{37} and DOCA-salt- and angiotensin II–induced hypertension,\textsuperscript{38} as well as pain, fever, and inflammation in animal models of these diseases.\textsuperscript{13,39} Conversely, global deletion of mPGES-1 does not disturb the balance between prothrombotic and anti-thrombotic prostaglandin production in vivo. On the basis of these results, pharmacological inhibitors of mPGES-1 are proposed as an alternative to inhibition of COX-2 in the management of patients with atherosclerosis, pain, and inflammatory diseases.\textsuperscript{40} The present results suggest that mPGES-1 in bone marrow–derived leukocytes negatively regulates the intensity of the inflammatory response to infarction and is necessary for physiological LV remodeling and recovery of LV contractile function after MI. Importantly, the effects of deletion of Ptges in bone marrow–derived leukocytes on cardiac physiology in mice may not be observed after administration of pharmacological inhibitors of the Ptges gene product mPGES-1 in animal models of MI or in patients. For example, 50% of mice lacking COX-2 develop diffuse cardiac fibrosis, but this finding is not observed in patients taking selective COX-2 inhibitors, possibly because of incomplete pharmacological inhibition of COX-2 in vivo. In addition, inhibition of COX-2 is cardioprotective in mice\textsuperscript{41} but results in adverse LV remodeling and LV rupture in a porcine MI model.\textsuperscript{32} These observations underscore the need for caution in extrapolating our findings in mice to humans and identify the need to confirm these results in clinically relevant MI models in larger animals.

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Disclosures

None.

References


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**CLINICAL PERSPECTIVE**

Millions of patients use nonsteroidal anti-inflammatory drugs (NSAIDS) to treat pain and inflammatory disorders. NSAIDS block the activity of cyclooxygenase-2 (COX-2), an enzyme that catalyzes the second of three steps in prostaglandin biosynthesis. Unfortunately, some COX-2 inhibitors are associated with an increased risk of myocardial infarction (MI) and stroke, possibly because of decreased production of antithrombotic eicosanoids, such as PGL2, combined with simultaneous unopposed production of prothrombotic thromboxane A2, an eicosanoid catalyzed via COX-1 in platelets. Microsomal prostaglandin E2 synthase-1 (mPGES-1) is downstream from COX-2 in the inducible PGE2 biosynthetic pathway. Inhibition or deletion of mPGES-1 decreases pain, fever and inflammation without increasing the propensity for thrombosis. Therefore, pharmacologic inhibitors of mPGES-1 may be a viable replacement for COX-2 inhibitors, and may not be associated with an increased risk of thrombotic cardiovascular events. We show that targeted deletion of mPGES-1 in bone marrow derived leukocytes that are recruited to the heart leads to left ventricular (LV) dilation, impaired LV systolic and diastolic function, adverse LV remodelling, and increased mortality after MI. These findings increase our understanding of the molecular events that control LV remodeling after MI, and demonstrate the importance of eicosanoid biosynthesis by inflammatory leukocytes in this process. However, caution is warranted in extrapolating the results of targeted deletion of mPGES-1 in bone marrow derived leukocytes in mice to the possible outcome of pharmacologic inhibition of mPGES-1 in clinical practice. Further studies, of mice and humans, are warranted to define the role of mPGES-1 in LV remodeling after MI.