Lack of Microsomal Prostaglandin E₂ Synthase-1 in Bone Marrow–Derived Myeloid Cells Impairs Left Ventricular Function and Increases Mortality After Acute Myocardial Infarction

Norbert Degousee, PhD*; Jeremy Simpson, PhD*; Shafie Fazel, MD, PhD*†; Klaus Scholich, PhD*; Denis Angoulvant, MD, PhD; Carlo Angioni, BSc; Helmut Schmidt, PhD†; Marina Korotkova, MD, PhD; Eva Stefanski, MSc; Xing-Hua Wang, MD; Thomas F. Lindsay, MD, MSc; Efrat Ofek, MD; Sandra Pierre, PhD; Jagdish Butany, MBBS, MS; Per-Johan Jakobsson, MD, PhD; Armand Keating, MD; Ren-Ke Li, MD, PhD; Matthias Nahrendorf, MD, PhD; Gerd Geisslinger, MD, PhD; Peter H. Backx, PhD; Barry B. Rubin, MD, PhD

Background—Microsomal prostaglandin E₂ synthase-1 (mPGES-1), encoded by the Ptges gene, catalyzes prostaglandin E₂ biosynthesis and is expressed by leukocytes, cardiac myocytes, and cardiac fibroblasts. Ptges⁻/⁻ mice develop more left ventricle (LV) dilation, worse LV contractile function, and higher LV end-diastolic pressure than 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 Ptges⁺/⁺ mice with Ptges⁺/⁺ bone marrow (BM⁺/⁺) and Ptges⁻/⁻ mice with 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 E₂ 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 E₂ 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

Editorial see p 2818

Clinical Perspective on p 2913

Prostaglandins, synthesized by the sequential action of phospholipase A₂ (PLA₂), cyclooxygenases (COX-1 and/or

Received November 28, 2011; accepted April 4, 2012.

From the Divisions of Vascular Surgery (N.D., E.S., T.F.L., B.B.R.), Cardiac Surgery (S.F., R.-K.L.), Cardiology (P.H.B.), and Pathology (E.O., J.B.), Peter Munk Cardiac Centre, and the Department of Medical Oncology & Hematology (X.-H.W., A.K.), Toronto General Hospital, University Health Network, Toronto, Canada; Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada (J.S., P.H.B.); Institut für Klinische Pharmakologie, Frankfurt am Main, Germany (K.S., C.A., H.S., S.P., G.G.); Division of Cardiology, Trousseau Hospital, Tours University Hospital Center and EA 4245, François Rabelais University, Tours, France (D.A.); Department of Medicine, Rheumatology Unit, Karolinska University Hospital, Stockholm, Sweden (M.K., P.-J.J.); and Center for Systems Biology, Massachusetts General Hospital and Harvard Medical School, Boston, MA (M.N.).

†Deceased.

*Dr Degousee, Simpson, Fazel, and Scholich contributed equally to this article.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.099754/-/DC1.

Correspondence to Barry B. Rubin, MD, PhD, Medical Director, Peter Munk Cardiac Centre, Division of Vascular Surgery, University Health Network, 200 Elizabeth St, EN6-222, Toronto, Ontario, Canada M5G–2C4. E-mail barry.rubin@uhn.on.ca

© 2012 American Heart Association, Inc.
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 Ptgse+/+ 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/1maJ 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, and indicated significant MI in both groups of mice (Figure 1B). At 28 days post-MI, LV volume (Figures 1C and 1D) and diameter (Table), measured after hyperkalemic arrest and constant-pressure in situ perfusion fixation, were 43% and 30% greater in BM−/− than in BM+/+ mice, respectively. Conversely, the volume of infarcted myocardium (Figures 1C and 1E) and the thickness of the interventricular septum (Table) were similar in BM+/+ and BM−/− mice 28 days after MI.

Next, we assessed LV dimensions and LV function in BM−/− and BM+/+ chimeras in vivo by 2-dimensional echocardiography and invasive hemodynamic monitoring. The decrease in LV fractional shortening in BM−/− and BM−/− mice 7 days after coronary ligation was comparable and indicated significant MI in both groups of mice (Figure 1F). Fractional shortening and +dP/dt (Figure 1G), indexes of LV systolic function, and −dP/dt and τ (Figures 1H and 1I), indicators of the diastolic properties of the LV, were all significantly worse in BM−/− than BM+/+ mice 28 days after MI. BM−/− mice also had higher LV diameter and LV volume at end systole but not end diastole, a lower stroke volume (Table), and higher LV end-diastolic pressure (Figure 1J) than 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-
ment Figure II), or myofibroblast formation (smooth muscle α-actin; online-only Data Supplement Figure III) between BM/H11001/H11001 and BM/H11002/H11002 mice after MI.

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.

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+/H11001/H11001 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, +dP/dt; 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.
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. 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+/- and BM-/- 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 chimeras, and the expression of these genes was higher in BM-/- than BM+/- 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 both chimeras but were not different between BM+/- and BM+/- mice (Figures 2D and 2E). Changes in keratinocyte-derived chemokine mRNA expression in the infarct were not identified after MI in these mice (Figure 2F). There was no difference in the expression of IL-1β, IL-1β 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-/- and BM+/- 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. Levels of MPO were significantly higher in the infarct of BM-/- than BM+/- 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-/- and BM+/- 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-/- than BM+/- 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-/- Mice After MI

Targeted deletion of mPGES-1 leads to alterations in the biosynthesis of multiple prostaglandins in macrophages, and macrophages are the most prominent inflammatory cells in the infarct zone 7 days after MI. To explore whether changes in prostaglandin concentrations may explain the differences in leukocyte recruitment between BM-/- and BM+/- 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 PGE2, thromboxane B2 (TxB2, a stable TxA2 metabolite), PGF2α, 6-keto-PGF1α (a stable PGI2 metabolite), and total prostaglandins were significantly higher in the infarct of BM-/- than

---

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

<table>
<thead>
<tr>
<th>BM+/- (n=14)</th>
<th>BM-/- (n=13)</th>
<th>BM+/- (n=13)</th>
<th>BM-/- (n=11)</th>
<th>BM+/- (n=10)</th>
<th>BM-/- (n=8)</th>
</tr>
</thead>
<tbody>
<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>
<td>3.3±0.1*</td>
<td>3.1±0.1*</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>
<td>3.9±0.09</td>
<td>4.0±0.09*</td>
</tr>
<tr>
<td>LV volume, mL‡</td>
<td>22±1.1</td>
<td>26±2.0</td>
<td>40±1.9*</td>
<td>45±3.1*</td>
<td>38±3.4*</td>
</tr>
<tr>
<td>LV volume, mL§</td>
<td>54±1.6</td>
<td>58±2.7</td>
<td>66±2.7*</td>
<td>66±3.6</td>
<td>69±3.7*</td>
</tr>
<tr>
<td>Stroke volume, mL‡</td>
<td>31±0.9</td>
<td>33±1.8</td>
<td>26±1.4</td>
<td>21±1.9*</td>
<td>31±2.4</td>
</tr>
<tr>
<td>Cardiac output, mL/min‡</td>
<td>15±0.6</td>
<td>16±0.8</td>
<td>13±0.5*</td>
<td>11±1.1*</td>
<td>17±1.2</td>
</tr>
<tr>
<td>Heart rate, bpm‡</td>
<td>491±9</td>
<td>491±15</td>
<td>490±11</td>
<td>533±13</td>
<td>542±14</td>
</tr>
<tr>
<td>LV volume, mL§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>13.7±0.7</td>
</tr>
<tr>
<td>LV diameter, mm§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>Interventricular septum, mm§</td>
<td>ND</td>
<td>ND</td>
<td>163±11</td>
<td>165±7</td>
<td>158±9</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min§</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.4±0.1</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+/- vs BM-/- mice.
‡In vivo 2-dimensional echocardiography.
§Ex vivo morphometric analysis.
$BM^{-/-}$ mice 7 but not 28 days after MI (Figures 3A through 3F). Similarly, PGE$_2$, PGD$_2$, TxB$_2$, 6 keto-PGF$_{1a}$, and total prostaglandin levels were higher in the viable myocardium adjacent to the infarct in $BM^{-/-}$ than in $BM^{+/+}$ 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, increased prostaglandin levels in the infarct and LV of $BM^{-/-}$ mice 7 but not 28 days after coronary ligation (Figure 3I). The 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.

To identify the molecular mechanisms that resulted in increased prostaglandin levels in the infarct and LV of $BM^{-/-}$ compared with $BM^{+/+}$ mice, we assessed prostaglandin biosynthetic enzyme gene expression in these chimeras after MI. Our studies focused on COX-1 and COX-2 because these enzymes catalyze the formation of PGH$_2$, the precursor of all prostaglandins, and we noted an increase in the level of mPGES-1 mRNA in the infarct was also higher in $BM^{-/-}$ mice 7 but not 28 days after MI (Figures 3G and 3H). 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).

COX-1 protein levels in the infarct were higher in $BM^{-/-}$ than in $BM^{+/+}$ mice 7 but not 28 days after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No change in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L). No difference in the level of COX-2 protein in the infarct between these chimeras after MI (Figures 3J and 3L).
expression in the heart 7 days after MI, because that was when differences in prostaglandin levels in the infarct (Figures 3A through 3F) and LV (online-only Data Supplement Figure VI, A through F) were identified between BM+/− and BM+/+ mice. Seven days after MI, COX-1 protein was present in the infarct, with apparently more intense staining in BM−/− than BM+/+ mice (Figures 4A and 4B), consistent with the immunoblot analysis (Figures 3J and 3K). Scant cells stained positively for COX-2 protein 7 days after MI (Figures 4A and 4B). Inflammatory cells in the infarct zone expressed mPGES-1 protein in BM+/− mice (Figure 4A), as previously observed in Ptges+/− mice after MI. In addition, elongated cells in the infarct zone of BM−/− mice also expressed mPGES-1 protein (Figure 4B). The identity of these cells is unknown, but they could not have been bone marrow–derived leukocytes, because all bone marrow–derived cells in BM−/− mice lacked the ability to express mPGES-1 (Figure 1A). Therefore, the cells that expressed mPGES-1 protein in BM−/− mice 7 days after MI must have been resident cardiac cells.

In contrast to the changes in prostaglandin biosynthetic enzyme gene expression in the infarcted myocardium (Figures 3G through 3I), there was no difference in COX-1, COX-2, mPGES-1, mPGES-2, or cPGES mRNA levels in the viable LV adjacent to the infarct in BM+/+ and BM−/− mice after MI (online-only Data Supplement Figure VII, A through E).

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.

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, cardiac fibroblasts have all of the enzymatic machinery necessary to catalyze PGE2 biosynthesis. In con-
Contrast, cardiomyocytes did not express mPGES-1 in BM+/− mice (Figure 4G) after MI. In BM+/− 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+/− mice 7 days after MI (Figure 4B) were most likely cardiac fibroblasts.

**Higher PGE2 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, to generate PGE2 in vitro was evaluated next. Treatment with IL-1β increased PGE2 production in vitro by bone marrow–derived mononuclear cells/macrophages and by cardiac fibroblasts from Ptges+/− mice (Figure 5A). On a per cell basis, cardiac fibroblasts produced 8.8-fold more PGE2 than bone marrow–derived mononuclear cells/macrophages after exposure to IL-1β in vitro (Figure 5A). Although the protocol used generated a cellular population that was highly enriched in cardiac fibroblasts (online-Data Supplement Methods), it is possible that other cells in this preparation also produced PGE2 in response to IL-1β.

Because PGH2 generated by macrophages recruited to the infarct may diffuse out of these cells and be metabolized to PGE2 by mPGES-1 in adjacent cardiac fibroblasts, we evaluated the effect of coculturing cardiac fibroblasts from Ptges+/− mice with mononuclear cells/macrophages from Ptges+/+ or Ptges−/− mice in vitro. There is no difference in the level of PGE2 in the supernatant of Ptges+/− cardiac fibroblasts after coculture with mononuclear cells/macrophages from either Ptges+/+ or Ptges−/− mice and treatment with vehicle or IL-1β 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, (2) inhibition or deletion of the PGE$_2$ receptor EP4 attenuates cardiomyocyte hypertrophy in vitro and in vivo, respectively, (3) Ptges$^{-/-}$ mice have lower levels of PGE$_2$ and less cardiac myocyte hypertrophy than Ptges$^{+/+}$ mice after MI, (4) Ptges$^{-/-}$ mice have an impaired cardiac hypertrophic response compared with Ptges$^{+/+}$ mice to angiotensin II infusion in vivo, 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, induction of COX-1 has been observed during inflammatory responses and cellular differentiation. It is possible that IL-1$\beta$ stimulates COX-1 expression in BM$^{-/-}$ mice after MI, because IL-1$\beta$ can stimulate COX-1 expression in cultured fibroblasts under certain conditions, and coronary ligation leads to higher IL-1$\beta$ 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, 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 $\approx$8.8-fold more PGE$_2$ per cell than mononuclear cells/macrophages in vitro with treatment with IL-1$\beta$ (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 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, granulation tissue formation, angiogenesis, and infarct healing. In addition, leukocyte-mediated oxidation reactions play a critical role in LV remodeling after MI.
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-α mRNA expression and leukocyte recruitment to the infarct (cf Figure 2) may explain the adverse LV remodeling that we observed in BM\textsuperscript{-/-} compared with BM\textsuperscript{+/+} mice after MI.

Inhibition of COX-2 leads to an imbalance of prothrombotic prostaglandins (increased TxA\textsubscript{2}) and antiithrombotic prostaglandins (decreased PGJ\textsubscript{2}) that is proposed to increase the risk of MI and stroke\textsuperscript{33} 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 isch-mia-reperfusion injury,\textsuperscript{35} plaque burden in fat-fed Ptges\textsuperscript{-/-} low-density lipoprotein receptor-deficient (LDLR\textsuperscript{-/-}) mice,\textsuperscript{36} aortic aneurysm formation,\textsuperscript{37} and DOCA-salt- and angioten-sin 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 antiithrombotic 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.

**Acknowledgments**

This study is dedicated to the memory of Shafie Fazel and Helmut Schmidt.

**Sources of Funding**

This study was supported by Canadian Institutes of Health Research grants (53297 to Dr Rubin, 14795 to Drs Li and Fazel), Swedish Medical Research Council, Swedish Rheumatism Association, King Gustav V 80 Years and Marianne and Marcus Wallenberg’s Foundation and Karolinska Institutet (Dr Jakobsson), and Deutsche Forschungsgemeinschaft DFG (GE 695) and Excellence Cluster 147 Cardiopulmonary System (ECCPS, Dr Geisslinger), Dr Keating is the Gloria and Seymour Epstein Chair in Cell Therapy and Transplantation at University Health Network. Dr Rubin is a Wylie Scholar in Academic Vascular Surgery, Foundation for Accelerated Vascular Research, San Francisco and the Peter Munk Cardiac Centre Medical Director Chair.

**Disclosures**

None.

**References**


Leukocyte mPGES-1 Regulates LV Remodeling After MI

Degousee et al


20. Colston JT, de la Rosa SD, Strader JR, Anderson MA, Freeman GL. 


**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 PGI2, 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.
Lack of Microsomal Prostaglandin E_{2} Synthase-1 in Bone Marrow-Derived Myeloid Cells Impairs Left Ventricular Function and Increases Mortality After Acute Myocardial Infarction

Norbert Degousee, Jeremy Simpson, Shafie Fazel, Klaus Scholich, Denis Angoulvant, Carlo Angioni, Helmut Schmidt, Marina Korotkova, Eva Stefanski, Xing-Hua Wang, Thomas F. Lindsay, Efrat Ofek, Sandra Pierre, Jagdish Butany, Per-Johan Jakobsson, Armand Keating, Ren-Ke Li, Matthias Nahrendorf, Gerd Geisslinger, Peter H. Backx and Barry B. Rubin

_Circulation._ 2012;125:2904-2913; originally published online May 15, 2012;
doi: 10.1161/CIRCULATIONAHA.112.099754

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/125/23/2904

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2012/05/14/CIRCULATIONAHA.112.099754.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/
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.

N. Degousee et al.

SUPPLEMENTAL MATERIAL

Supplemental Methods.

Immunoblotting, immunohistochemistry, MELC and real time PCR

Myeloperoxidase (MPO) antiserum for immunoblotting was from Upstate Biotechnology (Lake Placid, NY.) For immunohistochemical studies, antiserum against CD-31, $\alpha$-smooth muscle actin (SMA) and MPO (Abcam, Cambridge, MA) and CD-45 (BD PharMingen, San Jose, CA) were used.

The fluorescence tagged antibodies used for MELC included: polyclonal rabbit anti-mPGES-1 (Cayman Chemicals, Ann Arbor, MI), mouse monoclonal anti-COX-1 clone CX111 (Cayman Chemicals), mouse monoclonal anti-COX-2 clone AS66 (Acris, San Diego, CA), rat monoclonal fibroblast marker ER-TR7 (Santa Cruz, Santa Cruz, CA), mouse monoclonal anti-cardiac troponin-I clone 19C7 (Hytest, Turku, Finland) and 7-Aminoactinomycin (Calbiochem, Darmstadt, Germany).

Primer sequences for real-time RT-PCR analysis included IL-1$\beta$ forward: CTGTGTCTTTCCCGTGGACC, reverse: CAGCTCATATGGGTCCGACA; IL-1 receptor antagonist forward: TTGTGCAAGTCTGGAGATG, reverse: TTCTCAGAGCGGATGAAGGT; TNF-$\alpha$ forward: CCCCCAAGGGATGAGAAGTT, reverse: AGGGTCTGCGGCCAT
AGAACT; Keratinocyte-derived Chemokine (KC) forward: ACTGCA CCCAAACCGAAGT, reverse: TTCTCCGTTACTTGGGACA; Macrophage Inflammatory Protein-2α (MIP-2) forward: AGTGAACCTGCGCTGTCAATGC, reverse: AGGCAAACTTTT TGACCGCC and Monocyte Chemotactic Protein-1 (MCP-1) forward: CCAACTCTCAGTC AAGCCAGCTC, reverse: TTGGGATCATCTTGGCTGAA.

**Bone marrow transplantation and generation of chimeric mice**

Bone marrow was isolated from male Ptges+/+ or Ptges−/− mice killed by cervical dislocation. Female recipient Ptges+/+ mice were irradiated with 2 sub-lethal doses of 9.5 Gy (Gammacell 40 Cs γ-irradiation source), which induces agranulocytosis, injected with 8×10⁶ bone marrow cells from donor male mice (via the tail vein) and kept in micro-isolator cages for 10 weeks to allow full humoral reconstitution, as described. The efficiency of BMT was estimated by measuring the ratio of SRY (a gene located on the Y chromosome) to GAPDH DNA, by real time PCR.

**Isolation and co-culture of cardiac fibroblasts and bone marrow derived mononuclear cells/macrophages.**

Cardiac fibroblasts were isolated from Ptges+/+ mice, exactly as described. Mononuclear cells/macrophages were prepared from the bone marrow of Ptges+/+ and Ptges−/− mice. The femur and tibia were dissected, cut proximally and distally and flushed with ice-cold HBSS supplemented with 10 mM HEPES, pH 7.5. The eluant was centrifuged at 2,600 rpm for 30 min at 4°C over a three-layer Percoll gradient (52, 65, and 75%). The mononuclear cell/macrophage rich fraction was collected at the interface of the 52 and 65% layers, re-suspended in 1 ml of
HBSS and centrifuged at 1,500 rpm for 5 min at 4°C. Pelleted cells were re-suspended in 1 ml of IDMEM with 10% fetal bovine serum, counted and used immediately. This protocol yields a population of cells that is typically ~92% macrophages.22

Co-culture experiments were performed at a fibroblast to mononuclear cells/macrophage ratio of 1:5. Cardiac fibroblasts (passage 2-4) at 3×10^4/well were cultured for 1 day in IDMEM with 10% fetal bovine serum. After 24 hours, medium was replaced and 1.5×10^5 freshly isolated mononuclear cells/macrophages from the bone marrow of Ptges^{+/+} or Ptges^{−/−} mice were added to each well and cultured for 24 hours. Then, medium was changed to serum-free IDMEM containing 0.1% BSA and cells were stimulated with vehicle or murine IL-1β (PeproTech Inc., Rocky Hill, NJ) at 10 ng/ml. For PGE$_2$ analysis, the medium was removed after 24 hours, stored at -80° C and analyzed by ELISA (Cayman Chemical, Ann Arbor, MI).

**Statistical Analysis**

Data are presented as mean ± SEM. Analyses of data recorded at 1 time point were performed by 2-tailed, unpaired, Student t tests. Analyses of data recorded at several time points for 2 groups (Ptges^{+/+} mice with Ptges^{+/+} bone marrow, Ptges^{+/+} mice with Ptges^{−/−} bone marrow) were performed by 2-way ANOVA (to evaluate the effect of group, time, and group–time interactions); if significant, a Bonferroni correction for multiple comparisons was applied for post-hoc analysis between different time points or between different groups at the same time point. Survival after coronary ligation was assessed by a log-rank test. A value of $P<0.05$ was accepted as statistically significant. In total, 176 Ptges^{+/+} mice, 16 Ptges^{−/−} mice, 70 Ptges^{+/+} mice with Ptges^{+/+} bone marrow and 69 Ptges^{+/+} mice with Ptges^{−/−} bone marrow were used in this study.
Supplemental Figure 1.
Supplemental Figure 2.
A.

Baseline LV

28 days post MI

MI junction area

MI body of Infarct

BM^{+/+}

SMA

BM^{-/-}

x200

x200

x400

B.

C.

Supplemental Figure 3.
Supplemental Figure 4.
Supplemental Figure 5.
Supplemental Figure 6.
Supplemental Figure 7.
Supplemental Figure 8.
Supplemental Figure 10.
Supplemental Figure Legends.

Supplemental Figure 1. No difference in MMP-2 or MMP-9 mRNA expression in the infarct of BM+/+ and BM−/− mice after MI. Before as well as 7 and 28 days after coronary ligation, A, MMP-2 mRNA and B, MMP-9 mRNA levels in the infarct zone were measured by real time PCR and normalized to GAPDH mRNA. Open bars, BM+/+ mice; solid bars, BM−/− mice. a, P<0.05 baseline vs. 7 or 28 days post MI; b, P<0.05, Ptges+/−BM+/+ mice vs. Ptges+/−BM−/− mice at any time point. Data represent ≥ 6 independent experiments, assessed in duplicate, for each group.

Supplemental Figure 2. No difference in endothelial cell growth in the heart of BM+/+ and BM−/− mice after MI. Before and 28 days after coronary ligation, A, CD31 expression was assessed by immunohistochemical analysis (magnification, ×200 and ×400); B, CD31 protein levels (expressed as percent of positive stained cells within the area sampled) in the myocardium immediately adjacent to the infarct (junction area) and C, in the infarct zone, were measured by digital image analysis of immunohistochemical staining using the Color Deconvolution image analysis tool (Aperio ScanScope software, Aperio Technologies, Inc) (Vista, California). Open bars, BM+/+ mice; solid bars, BM−/− mice. a, P<0.05 baseline vs. 7 or 28 days post MI; b, P<0.05,
BM\(^{+/+}\) mice vs. BM\(^{-/-}\) mice at any time point. Scale bars: 100 \(\mu\)m. Data represent \(\geq 6\) independent experiments for each group.

Supplemental Figure 3. No difference in myofibroblast formation in the heart of BM\(^{+/+}\) and BM\(^{-/-}\) mice after MI. Before and 28 days after coronary ligation, A, smooth muscle \(\alpha\)-actin (SMA) expression was assessed by immunohistochemical analysis (magnification, \(\times 200\) and \(\times 400\)); B, SMA protein levels (expressed as percent of positive stained cells within the area sampled) in the myocardium immediately adjacent to the infarct (junction area) and C, in the infarct zone, were measured by digital image analysis of immunohistochemical staining using the Color Deconvolution image analysis tool (Aperio ScanScope software, Aperio Technologies, Inc) (Vista, California). Open bars, BM\(^{+/+}\) mice; solid bars, BM\(^{-/-}\) mice. a, \(P<0.05\) baseline vs. 7 or 28 days post MI; b, \(P<0.05\), BM\(^{+/+}\) mice vs. BM\(^{-/-}\) mice at any time point. Scale bars: 100 \(\mu\)m. Data represent \(\geq 6\) independent experiments for each group.

Supplemental Figure 4. Similar inflammatory response in the viable LV remote from the infarct in BM\(^{+/+}\) and BM\(^{-/-}\) mice after MI. Ratio of A, IL-1 \(\beta\); B, IL-1 \(\beta\) receptor antagonist; C, TNF-\(\alpha\), D, MCP-1; E, MIP-2 and F, KC to GAPDH mRNA in the LV remote from the infarct before and 7 and 28 days after MI. G, representative immunoblot analysis of MPO and GAPDH protein from four separate BM\(^{+/+}\) and BM\(^{-/-}\) mice, and H, densitometric analysis of MPO
protein, Open bars indicate $BM^{+/+}$ mice, solid bars, $BM^{-/-}$ mice. a, P<0.05 baseline vs. 7 or 28 days post MI, $BM^{+/+}$ or $BM^{-/-}$ mice; b, P<0.05, $BM^{+/+}$ vs. $BM^{-/-}$ mice at any time point. Data represent ≥ 6 independent experiments for each group.

Supplemental Figure 5. Increased MPO levels in $BM^{-/-}$ mice after MI. A, Immunohistochemical analysis of MPO (magnification, ×200) and B, MPO percentage of MPO positive cells per high power field (indicated by $\rightarrow$) 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 post MI, $BM^{+/+}$ or $BM^{-/-}$ mice; b, P<0.05, $BM^{+/+}$ vs. $BM^{-/-}$ mice at any time point. Scale bars: 100 µm. Data represent ≥ 6 independent experiments for each group.

Supplemental Figure 6. Increased eicosanoid levels in the viable LV remote from the zone of infarction in $BM^{+/+}$ vs. $BM^{-/-}$ mice after MI. Levels of A, PGE$_2$; B, PGD$_2$; C, thromboxane B$_2$; D, PGF$_{2\alpha}$; E, 6 keto-PGF$_{1\alpha}$ and F, total prostaglandin levels (expressed as pg/mg of tissue) in viable myocardium, measured by LC-MS/MS 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 post MI; b, P<0.05, $BM^{+/+}$ mice vs. $BM^{-/-}$ mice at any time point. Data represent ≥ 12 independent experiments for each group.
Supplemental Figure 7. No difference in mPGES-1, mPGES-2, cPGES, COX-1 or COX-2 mRNA levels in the viable LV remote from the infarct in BM\(^{+/+}\) vs. BM\(^{-/-}\) mice after MI. Before as well as 7 and 28 days after coronary ligation A, mPGES-1; B, mPGES-2; C, cPGES; D, COX-1 and E, COX-2 mRNA were measured by real time PCR and normalized to GAPDH mRNA. Open bars indicate BM\(^{+/+}\) mice; solid bars, BM\(^{-/-}\) mice. a, P<0.05 baseline vs. 7 or 28 days post MI; b, P<0.05, BM\(^{+/+}\) mice vs. BM\(^{-/-}\) mice at any time point. Data represent ≥ 6 independent experiments for each group.

Supplemental Figure 8. mPGES-1 co-localizes with COX-1, COX-2 and fibroblasts but not cardiac myocytes in Ptges\(^{+/+}\) mice after MI. Twenty eight days after coronary ligation, the expression of A, mPGES-1, and co-localization of this protein with B, COX-1, C, COX-2, D, the fibroblast marker ER-TR7 and E, the cardiomyocyte marker troponin-I was assessed by MELC in Ptges\(^{+/+}\) mice. In the second column, the label “merge” identifies where mPGES-1 co-localizes with COX-1, COX-2, ER-TR7 or Troponin I. The area within the black rectangle in the Diff-Quick image for the Ptges\(^{+/+}\) mice is shown magnified in the mPGES-1, COX-1, COX-2, fibroblast and troponin-I images (magnification, ×200). The area within the white rectangle in the mPGES-1 image is shown magnified (×2) in the upper left corner of the merged images for COX-1, COX-2 and fibroblasts. Scale bars: (Diff-Quick) 200 \(\mu\)m; (mPGES-1) 50 \(\mu\)m; (white rectangle) 25 \(\mu\)m. Images are representative of 5 independent studies.
Supplemental Figure 9. Failure to detect mPGES-1 expression in \textit{Ptges}^{-/-} mice after MI.

Twenty eight days after coronary ligation, the expression of A, mPGES-1, and co-localization of this protein with B, COX-1, C, COX-2, D, ER-TR7 and E, troponin-I was assessed by MELC in \textit{Ptges}^{-/-} mice. In the second column, the label “merge” identifies where mPGES-1 co-localizes with COX-1, COX-2, ER-TR7 or Troponin I. The area within the black rectangle in the Diff-Quick image for the \textit{Ptges}^{-/-} mice is shown magnified in the mPGES-1, COX-1, COX-2, fibroblast and troponin-I images (magnification, \( \times 200 \)). The area within the white rectangle in the mPGES-1 image is shown magnified (\( \times 2 \)) in the upper left corner of the merged images for COX-1, COX-2 and fibroblasts. Scale bars: (Diff-Quick) 200 \( \mu \)m; (mPGES-1) 50 \( \mu \)m; (white rectangle) 25 \( \mu \)m. Images are representative of 5 independent studies.

Supplemental Figure 10. COX-1 and COX-2 co-localize with fibroblasts in \textit{BM}^{+/+} and \textit{BM}^{-/-} mice after MI. Twenty eight days after coronary ligation, co-localization of COX-1 and COX-2 with ER-TR7 was assessed by MELC in \textit{BM}^{+/+} and \textit{BM}^{-/-} mice. In the bottom panels, the label “merge” (D) identifies where mPGES-1 (A) co-localizes with COX-1 and COX-2 (B), and ER-TR7 (C). The area within the black rectangle in the Diff-Quick image for \textit{BM}^{+/+} and \textit{BM}^{-/-} mice is shown magnified in the mPGES-1, COX-1, COX-2, and fibroblast images (magnification, \( \times 200 \)). The area within the white rectangle in the mPGES-1 image is shown magnified (\( \times 2 \)) in the upper left corner of the merged images (D) for COX-1, COX-2 and fibroblasts. Scale bars: (Diff-Quick) 200 \( \mu \)m; (mPGES-1) 50 \( \mu \)m; (white rectangle) 25 \( \mu \)m. Images are representative of 6 independent studies.