

# Lp-PLA<sub>2</sub> Antagonizes Left Ventricular Healing After Myocardial Infarction by Impairing the Appearance of Reparative Macrophages

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**Background**—Healing after myocardial infarction (MI) involves the biphasic accumulation of inflammatory Ly-6C<sup>high</sup> and reparative Ly-6C<sup>low</sup> monocytes/macrophages. Excessive inflammation disrupts the balance between the 2 phases, impairs infarct healing, and contributes to left ventricle remodeling and heart failure. Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), a member of the phospholipase A<sub>2</sub> family of enzymes, produced predominantly by leukocytes, participates in host defenses and disease. Elevated Lp-PLA<sub>2</sub> levels associate with increased risk of cardiovascular events across diverse patient populations, but the mechanisms by which the enzyme elicits its effects remain unclear. This study tested the role of Lp-PLA<sub>2</sub> in healing after MI.

**Methods and Results**—In response to MI, Lp-PLA<sub>2</sub> levels markedly increased in the circulation. To test the functional importance of Lp-PLA<sub>2</sub>, we generated chimeric mice whose bone marrow-derived leukocytes were Lp-PLA<sub>2</sub>-deficient (*bmLp-PLA<sub>2</sub><sup>-/-</sup>*). Compared with wild-type controls, *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice subjected to MI had lower serum levels of inflammatory cytokines tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6, and decreased number of circulating inflammatory myeloid cells. Accordingly, *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice developed smaller and less inflamed infarcts with reduced numbers of infiltrating neutrophils and inflammatory Ly-6C<sup>high</sup> monocytes. During the later, reparative phase, infarcts of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice contained Ly-6C<sup>low</sup> macrophages with a skewed M2-prone gene expression signature, increased collagen deposition, fewer inflammatory cells, and improved indices of angiogenesis. Consequently, the hearts of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice healed more efficiently, as determined by improved left ventricle remodeling and ejection fraction.

**Conclusions**—Lp-PLA<sub>2</sub> augments the inflammatory response after MI and antagonizes healing by disrupting the balance between inflammation and repair, providing a rationale for focused study of ventricular function and heart failure after targeting this enzyme acutely in MI. (*Circ Heart Fail.* 2015;8:980-987. DOI: 10.1161/CIRCHEARTFAILURE.115.002334.)

**Key Words:** heart failure ■ inflammation ■ macrophages ■ monocytes ■ myocardial infarction

Myocardial infarction (MI) is a leading cause of death worldwide.<sup>1</sup> Although the case fatality rate of MI has declined, survival with development of long-term left ventricular (LV) dysfunction because of cumulative ischemic myocardial damage has added to the growing epidemic burden of chronic heart failure. The human, social, and economic consequences of chronic ischemic cardiomyopathy present a major challenge and unmet medical need. Patients who initially survive MI must overcome a major obstacle: ischemia damages the heart, and effective cardiac repair likely requires

a precise balance between removal of debris and formation of a scar that is compatible with heart function. MI survivors frequently develop heart failure; although many therapeutics in current use have proven beneficial, the high residual morbidity and mortality presents an urgent problem that requires a better understanding of the disease's pathophysiology.

## Clinical Perspective on p 987

During the past several years, neutrophils, monocytes, and macrophages have emerged as consequential to the

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inflammatory and healing process that occurs after MI.<sup>2</sup> We now understand that ischemic injury triggers the accumulation of these myeloid cells in the infarcted myocardium.<sup>3,4</sup> Shortly after onset of ischemia, large numbers of neutrophils and inflammatory Ly-6C<sup>high</sup> monocytes infiltrate the infarcted myocardium and produce interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$ . Within 4 to 5 days, Ly-6C<sup>high</sup> monocytes give rise to Ly-6C<sup>low</sup> reparative macrophages,<sup>3</sup> which potentiate healing via vascular endothelial growth factor, transforming growth factor- $\beta$ , and IL-10. The 2 phases comprising inflammatory Ly-6C<sup>high</sup> monocyte recruitment and reparative Ly-6C<sup>low</sup> macrophage differentiation are essential to post-MI recovery; their perturbation (ie, in the context of comorbidities) leads to impaired heart function and heart failure.<sup>3,4</sup>

Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), a member of the phospholipase A<sub>2</sub> family of enzymes, hydrolyses glycerophospholipids. The ensuing enzymatic reactions frequently generate metabolic signaling molecules with a multitude of biological actions. For example, by hydrolyzing phosphatidylcholine at C2 of the glycerol backbone, Lp-PLA<sub>2</sub> produces lysophosphatidylcholine,<sup>5</sup> which fosters oxidative stress, affects vascular smooth muscle cell proliferation, and increases tissue accumulation of macrophages.<sup>6</sup> Several observational studies showed that Lp-PLA<sub>2</sub> levels correspond with future cardiovascular events, such as acute MI and sudden cardiac death.<sup>7-9</sup> Although preliminary studies reported reduced development of advanced coronary atherosclerosis<sup>10</sup> or stabilization of the necrotic core size<sup>11</sup> with selective inhibition of Lp-PLA<sub>2</sub> by the inhibitor darapladib, 2 recently completed phase III trials did not provide evidence in favor of inhibiting Lp-PLA<sub>2</sub> in cardiovascular disease.<sup>12</sup> Neither study focused on LV function nor chronic heart failure end points. Moreover, it remains unclear whether Lp-PLA<sub>2</sub> participates in the inflammatory and reparative phases that characterize the innate immune response shortly after MI; these pathways likely influence LV remodeling and the development of chronic ischemic cardiomyopathy. This study sought to evaluate whether Lp-PLA<sub>2</sub> participates in infarct healing and HF after MI.

## Methods

Further details are available in the Data Supplement.

### Animals and Animal Experiments

Eight- to ten-week-old female C57BL6/J (wild-type [WT]) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Lp-PLA<sub>2</sub>-deficient mice (*Lp-PLA<sub>2</sub><sup>-/-</sup>*) were kindly provided by Glaxosmithkline Pharmaceuticals Ltd (King of Prussia, PA). All protocols were approved by the Animal Review Committee at Massachusetts General Hospital. C57BL6/J mice were lethally irradiated and reconstituted with WT and *Lp-PLA<sub>2</sub><sup>-/-</sup>* bone marrow to generate respective chimeric mice. The chimeras had normal leukocyte counts and exhibited no obvious abnormalities, consistent with the Lp-PLA<sub>2</sub>-deficient mice.<sup>13</sup> MI was induced by permanent ligation of the left anterior descending artery. We observed no differences in mortality between the groups.

### Lp-PLA<sub>2</sub>/PAF Acetyl-Hydrolase Activity Assay

The PAF hydrolase activity assay was performed as previously described<sup>14</sup> with modifications using [<sup>3</sup>H]PAF (Platelet Activating Factor, 1-O-Hexadecyl-[Acetyl-<sup>3</sup>H(N)]-, Hexadecyl PAF) as a substrate. Unlabeled PAF (1-O-Hexadecyl-2-O-acetyl-sn-glycero-

3-phosphorylcholine) was purchased from Enzo Life Sciences, 1-O-Hexadecyl-2-O-[Acetyl-<sup>3</sup>H(N)]-, Hexadecyl PAF, [acetyl-<sup>3</sup>H]-, (250 $\mu$ Ci (9.25MBq) was purchased from Perkin Elmer, and Bio-Safe II was purchased from Research Products International Corp, Mount Prospect, IL.

### Histology

Murine hearts were embedded in Tissue-Tek O.C.T compound (Sakura Finetek) and prepared for sectioning and staining.

### Flow Cytometry and Flow-Assisted Cell Sorting

Antibodies used for flow cytometry are listed in the Data Supplement. Data were acquired on a BD LSRII and analyzed with FlowJo. Cells were sorted with BD AriaII.

### Reverse Transcription Polymerase Chain Reaction

RNA was isolated from sorted cells with the RNeasy Micro Kit (Qiagen). Quantitative real-time TaqMan polymerase chain reaction was run on a 7500 PCR thermal cycler (Applied Biosystems).

### Magnetic Resonance Imaging

Magnetic resonance imaging was performed on days 1 and 21 after permanent coronary ligation as described previously.<sup>3</sup> We obtained cine images of the LV short axis by using a 7 Tesla horizontal bore Pharmascan (Bruker) and a custom-built mouse cardiac coil (Rapid Biomedical). Late gadolinium enhancement was performed on day 1 to determine infarct size. Acquisition was done as described previously.<sup>15</sup> Images were analyzed using the software Segment (<http://segment.heiberg.se>). The end-diastolic volume, end-systolic volume, ejection fraction, LV volume, heart rate, and cardiac output were measured.

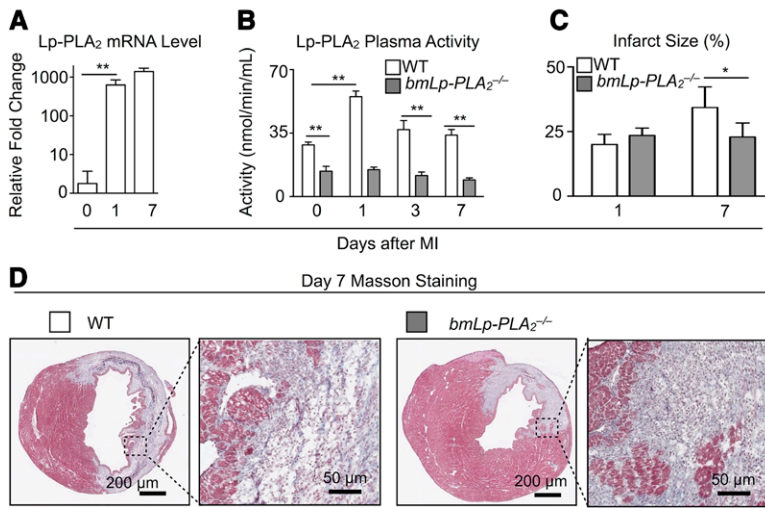
### Statistics

Results are shown as mean $\pm$ SEM. Unpaired Student *t* test was applied to evaluate differences between 2 groups. One-way ANOVA with post hoc Tukey multiple comparisons test was performed when comparing >2 groups between days, because different mice were euthanized on each time points for organ harvest. *P* values  $\leq 0.05$  denote significant changes.

## Results

### Expression of Lp-PLA<sub>2</sub> After MI and Its Role on Healing

To elucidate whether Lp-PLA<sub>2</sub> participates in healing after MI, we first measured Lp-PLA<sub>2</sub> expression and serum activity in steady state and after MI. Lp-PLA<sub>2</sub> mRNA expression by real-time polymerase chain reaction increased in the infarcts of WT mice as early as 1 day after MI (Figure 1A), suggesting Lp-PLA<sub>2</sub> may participate in myocardial ischemic injury. Concordantly, Lp-PLA<sub>2</sub> activity was also increased shortly after MI (Figure 1B). To define the role of Lp-PLA<sub>2</sub> in hematopoietic cells in the pathophysiology of acute MI, we generated chimeric mice by irradiating and reconstituting WT mice with bone marrow either from WT or from *Lp-PLA<sub>2</sub><sup>-/-</sup>* (*bmLp-PLA<sub>2</sub><sup>-/-</sup>*) mice. In comparison with WT mice, *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice had lower Lp-PLA<sub>2</sub> activity at steady state, and this activity did not change after MI (Figure 1B). These findings establish leukocytes as major sources of Lp-PLA<sub>2</sub> in response to MI. We then evaluated infarct healing and demonstrated a significant decrease in infarct size in *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice 7 days after MI compared with WT (Figure 1C and 1D).

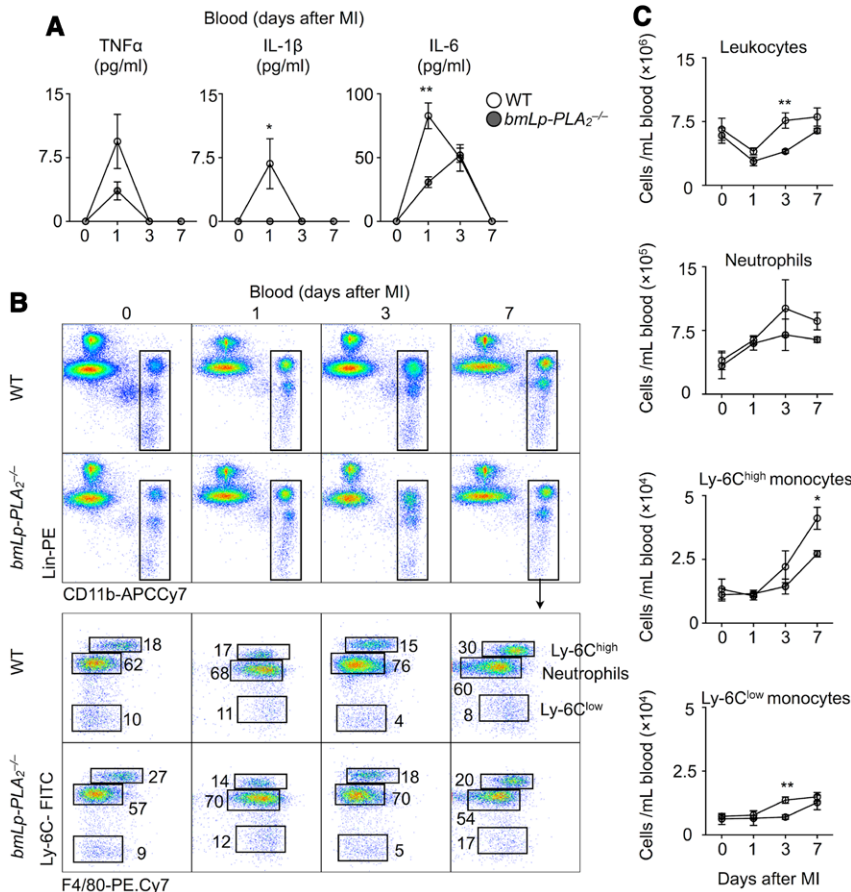


**Figure 1.** Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) activity and mRNA expression during myocardial infarction. **A**, mRNA levels quantified by real-time polymerase chain reaction in wild-type (WT) mice at the indicated time points after myocardial infarction (MI). Day 0 represents the steady-state mice. Results are presented as mean±SD, \*\**P*≤0.01, n=5 to 10 per group. **B**, Plasma Lp-PLA<sub>2</sub> activity in chimera WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice at steady state and in *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice at indicated time points after MI. \*\**P*≤0.01, n=5 to 10 per group. **C**, Quantification of infarct size on days 1 and 7 after MI in WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice (left). \**P*≤0.05, n=4 to 6 per group. **D**, Representative images of infarct size on day 7 after MI were shown in both WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice.

**Lp-PLA<sub>2</sub> Influences Systemic Inflammation**

Coronary occlusion stimulates an inflammatory response characterized by cytokine and chemokine production, and leukocyte recruitment to the heart. Because Lp-PLA<sub>2</sub> participates in inflammation, we assessed the effect of Lp-PLA<sub>2</sub> deficiency after MI. Serum concentrations of inflammatory cytokines tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-6 increased dramatically in WT mice on day 1 after MI, and eventually declined to undetectable amounts on day 7. In contrast, *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice showed only moderately elevated tumor

necrosis factor- $\alpha$  and IL-6, and negligibly increased IL-1 $\beta$ , demonstrating a diminished inflammatory response in the absence of Lp-PLA<sub>2</sub> (Figure 2A). Time-course profiling of circulating leukocytes after MI revealed that both WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice augmented inflammatory myeloid cells (neutrophils and Ly-6C<sup>high</sup> monocytes), but compared with WT mice, *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice had fewer neutrophils and Ly-6C<sup>high</sup> cells in blood at days 3 and 7, indicating Lp-PLA<sub>2</sub> contributes to the systemic inflammation after MI (Figure 2B and 2C).



**Figure 2.** Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) moderately affects systemic inflammation. **A**, ELISA analysis of plasma levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$  and IL-6 in wild-type (WT) and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice at the indicated time points after myocardial infarction (MI). **B**, Representative flow cytometric images of leukocyte profiling of blood in WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice. **C**, Quantification of total leukocytes, neutrophils, Ly-6C<sup>high</sup> monocytes, Ly-6C<sup>low</sup> monocytes in the blood. Results in 1 of 3 experiments with similar patterns are presented as mean±SEM, \**P*≤0.05, \*\**P*≤0.01 (WT vs *bmLp-PLA<sub>2</sub><sup>-/-</sup>*) vs n=5 per group.



### Lp-PLA<sub>2</sub> Impairs the Appearance of Reparative Macrophages

The myocardium displays a biphasic monocyte and macrophage response during MI.<sup>3,4</sup> In the first phase, inflammatory Ly-6C<sup>high</sup> monocytes infiltrate the ischemic myocardium from the blood and participate in inflammation. In the second phase, reparative Ly-6C<sup>low</sup> macrophages contribute to collagen deposition and scar formation. We profiled leukocytes in the myocardium in the steady state and 1, 3, and 7 days after MI in both WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice. The infarcts of both strains accumulated neutrophils, which peaked on day 1, Ly-6C<sup>high</sup> monocytes, which peaked on day 3, and Ly-6C<sup>low</sup> macrophages, which peaked on day 7 (Figure 3A and 3B). This finding agrees with our previous observations.<sup>3,4</sup> Yet, for nearly every peak, infarcts of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice accumulated only half the number of cells (neutrophils on day 1 and Ly-6C<sup>high</sup> monocytes on day 3) compared with WT controls, consistent with our observations in the blood (Figure 2), and affirming that Lp-PLA<sub>2</sub> aggravates inflammation.

Aside from determining the number of cells that accumulate (quantity), macrophage activity (quality) is an essential measure of the cells' impact on inflammation and repair. We asked whether Lp-PLA<sub>2</sub> shapes macrophage function by measuring expression of signature M1/M2 genes in sorted cardiac macrophages. In comparison with WT macrophages, *Lp-PLA<sub>2</sub><sup>-/-</sup>* macrophages exhibited higher expression of mRNAs that encode M2-associated genes (Arg, IL-10, CD36, and Fizz) and lower levels of those corresponding to M1-associated genes (MMP-3, MMP-9, TLR9, and TLR4; Figure 3C). These data are consistent with the idea that lysophosphatidylcholine, a product of Lp-PLA<sub>2</sub>, potentiates an

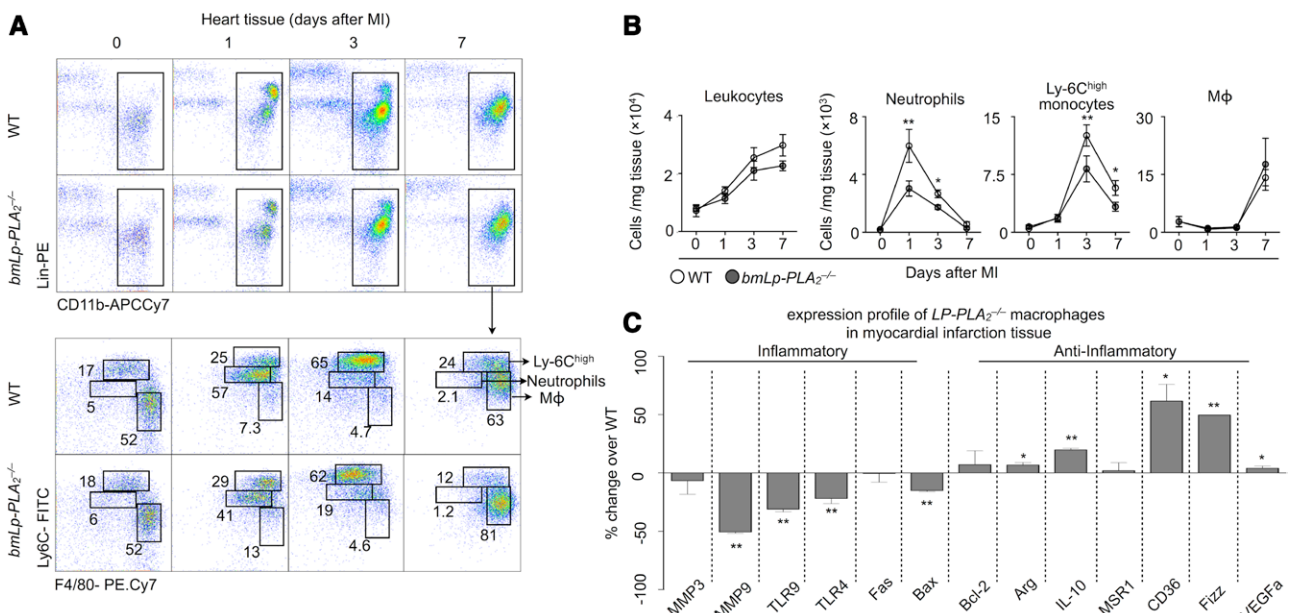
M1-like macrophage phenotype.<sup>16</sup> Together, these data show that Lp-PLA<sub>2</sub> promotes recruitment of inflammatory myeloid cells and delays the appearance of reparative macrophages in the ischemic myocardium.

### Lp-PLA<sub>2</sub> Retards Healing After MI

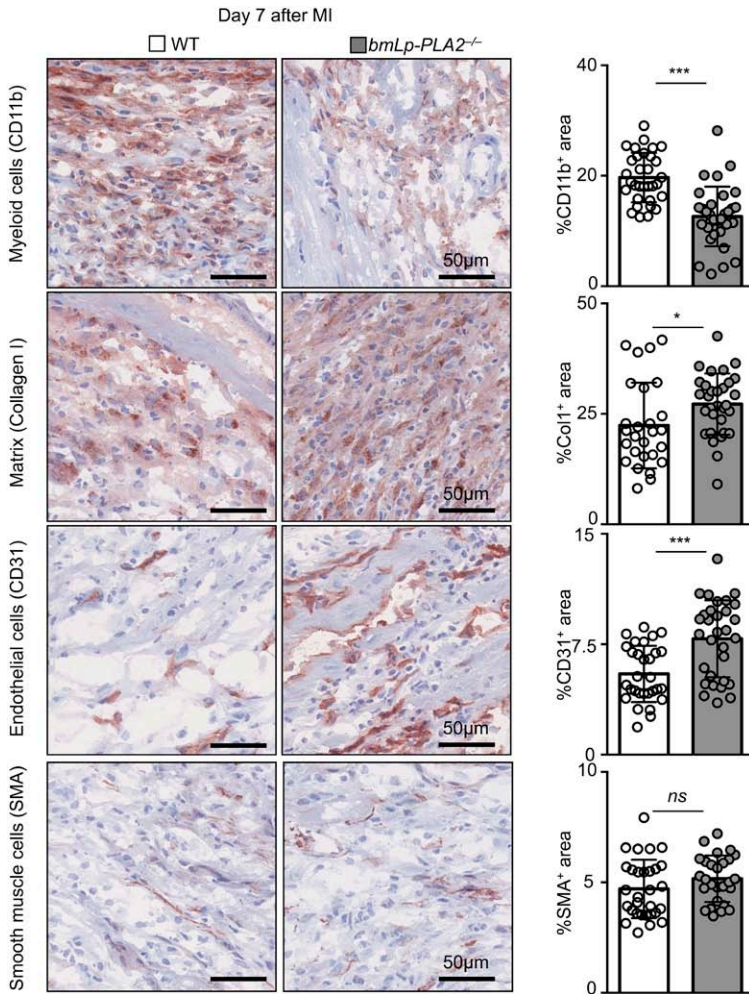
The differences in leukocyte recruitment between WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice prompted us to determine whether the absence of Lp-PLA<sub>2</sub> affects the repair of the ischemic myocardium. To this end, we profiled myeloid cell infiltration, extracellular matrix deposition, neovascularization, and smooth muscle cell accumulation by immunohistochemistry (Figure 4). Compared with WT controls, the myocardium of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice accumulated fewer myeloid CD11b<sup>+</sup> cells, indicating less severe inflammation. Infarcts of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice also had larger regions of extracellular matrix deposition, as evidenced by higher percentage of collagen I<sup>+</sup> areas (24% versus 32%), larger CD31<sup>+</sup> areas (5% versus 11%), suggesting improved neovascularization of the heart, but no changes in the number of smooth muscle actin<sup>+</sup> myofibroblasts (α-smooth muscle actin+area). Collectively, the results of histological examination demonstrate more effective healing in the absence of Lp-PLA<sub>2</sub>. These results demonstrate that Lp-PLA<sub>2</sub> inhibits the resolution of inflammation after MI.

### Improved Heart Function in the Absence of Lp-PLA<sub>2</sub>

To test whether inflammation mediated by Lp-PLA<sub>2</sub> after MI translated to impaired heart function, we performed magnetic resonance imaging in vivo in WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice. In the steady state, we detected no differences in cardiac function



**Figure 3.** Attenuated inflammatory response in *bmLp-PLA<sub>2</sub><sup>-/-</sup>* myocardial infarct tissue. **A**, Representative images for flow cytometric analysis of myocardial infarction (MI) tissue cell suspensions at the indicated time points after MI in wild-type (WT) and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice. **B**, Flow cytometry based quantification of neutrophil, monocyte and MΦ (macrophage) numbers in MI tissue of WT versus *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice before and 1, 3, and 7 days post MI. Results in one of three experiments with similar patterns are presented as mean±SEM, \* *P*≤0.05, n = 4 per group. **C**, Gene expression profiling of WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* macrophages sorted from MI tissue 7 days after permanent left anterior descending artery ligation. Results are presented as mean±SEM percent change of marker expression in *Lp-PLA<sub>2</sub><sup>-/-</sup>* compared with WT control mice, \**P*≤0.05, \*\**P*≤0.01, n=5 per group. IL indicates interleukin; and VEGF, vascular endothelial growth factor.



**Figure 4.** Improved cardiac remodeling in *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice 7 days after myocardial infarction. Immunohistochemical staining of myocardial infarction (MI) tissue for CD11b, Collagen 1 (Col1), CD31, and nonvascular smooth muscle  $\alpha$  actin (SMA) in wild-type (WT) and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* chimeric mice 7 days post MI. Quantification of 10 randomly selected fields of view per mouse (5 mice). Results are presented as mean $\pm$ SEM, \* $P$  $\leq$ 0.05, \*\*\* $P$  $\leq$ 0.001, n=50 fields of view per group.

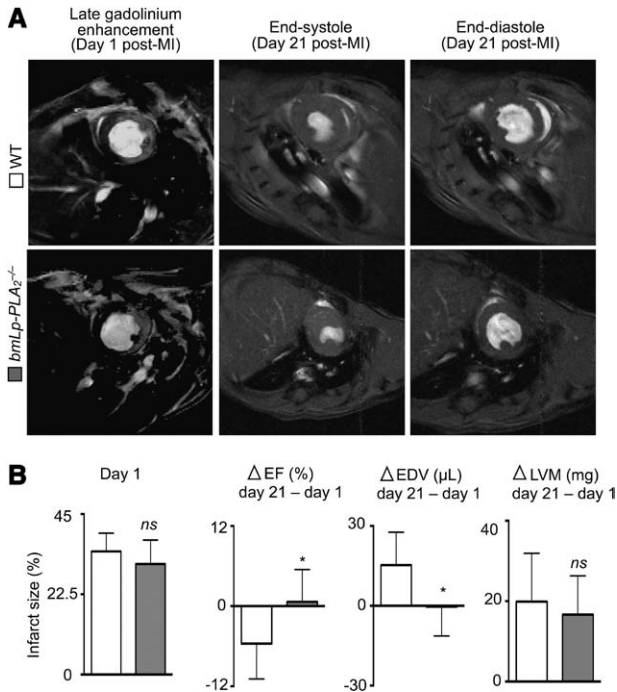
between WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice. After permanent coronary artery ligation, the end-diastolic volume and end-systolic volume, ejection fraction, LV volume, heart rate, and cardiac output were measured in individual mice on days 1 and 21 after MI. Late gadolinium enhancement was performed on day 1 to determine infarct size (Figure 5A and 5B; Table I in the Data Supplement). The infarct sizes were similar on day 1 in both groups, excluding a potential surgical bias (Figure 5B). Although LV volume increased similarly between the 2 groups on day 21, increased end-diastolic volume was only observed in WT mice, indicating more favorable remodeling in mice lacking Lp-PLA<sub>2</sub>. Moreover, compared with day 1, ejection fraction at day 21 diminished in WT mice but increased modestly in *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice, suggesting improved recovery of heart function in the absence of Lp-PLA<sub>2</sub>. Overall, the data show that Lp-PLA<sub>2</sub> aggravates LV remodeling and impairs LV function after MI.

### Discussion

Recruitment of neutrophils and Ly-6C<sup>high</sup> monocytes into the infarcted myocardium and the subsequent generation of reparative macrophages from Ly-6C<sup>high</sup> monocytes contribute to necrotic debris clearance, matrix deposition, granulation tissue formation, and angiogenesis. Perturbations in the inflammatory response impair infarct healing and promote

heart failure.<sup>17-19</sup> This study shows that deficiency of Lp-PLA<sub>2</sub> on hematopoietic cells attenuates systemic inflammation after MI, impairs leukocyte infiltration into infarcts, and enhances generation of reparative Ly-6C<sup>low</sup> macrophages, leading to less adverse LV remodeling and improved recovery of LV function. Together, the data show that Lp-PLA<sub>2</sub> modulates inflammation after MI and suggest that targeting of Lp-PLA<sub>2</sub> might lessen LV dysfunction and the development of chronic heart failure after MI.

Monocyte-derived macrophages can produce substantial Lp-PLA<sub>2</sub>,<sup>20</sup> and in the mouse, Lp-PLA<sub>2</sub> is expressed almost exclusively by myeloid cells (www.immgen.org). Previous studies have identified macrophage Lp-PLA<sub>2</sub> expression at both the mRNA and the protein levels in human and rabbit aortic lesions.<sup>21</sup> Plaques with characteristics of vulnerable and ruptured atheromata, but not early lesions, contain abundant Lp-PLA<sub>2</sub>.<sup>22</sup> Therefore, extensive efforts have been taken to prevent atherosclerosis-related coronary heart disease by inhibiting Lp-PLA<sub>2</sub> and thus promoting plaque stability.<sup>12</sup> But Lp-PLA<sub>2</sub> may participate in coronary heart disease by modulating inflammation independent of effects on the plaque itself. For example, our data showed that Lp-PLA<sub>2</sub> rose dramatically during MI. As a phospholipase, increased Lp-PLA<sub>2</sub> hydrolyzes phospholipids of oxidatively damaged cells or lipoproteins. Lp-PLA<sub>2</sub> hydrolyzes oxidized phosphatidylcholine,



**Figure 5.** Lp-PLA<sub>2</sub> aggravates LV dysfunction after myocardial infarction. **A**, Representative MRI with late gadolinium enhancement (left panels) on day 1 after permanent left anterior descending artery ligation, and end-systole (middle panels) and end-diastole (right panels) on day 21 of infarcted hearts from wild-type (WT) and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice, respectively. **B**, Quantification of individual changes ( $\Delta$ ) in heart parameters from day 1 to day 21 post myocardial infarction (MI; day 21–day 1). Results are presented as mean $\pm$ SD percent change of marker expression in WT and *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice, respectively. \*  $P \leq 0.05$ ,  $n \geq 7$  per group. EDV indicates end-diastolic volume; EF, ejection fraction; and LVM, left ventricle volume.

generating lysophosphatidylcholine and oxidized fatty acids that are capable of acting as monocyte chemoattractants as well as maintaining macrophages in a proinflammatory phenotype.<sup>6,16,23</sup> Lp-PLA<sub>2</sub> can also contribute to inflammation by interfering with phagocytic clearance of apoptotic cells, for example, neutrophils, via cleavage and removal of oxidized phosphatidylserine, a known eat-me signal.<sup>24</sup> Consistent with this hypothesis, we found that *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice had milder inflammatory responses as well as fewer neutrophils and Ly-6C<sup>high</sup> monocytes accumulating in the myocardium. This reduced number of inflammatory leukocytes ameliorated adverse LV remodeling and improved heart function recovery. Therefore, our data strongly support a role for Lp-PLA<sub>2</sub> in increased risk of heart failure by directly promoting systemic and local myocardial inflammatory responses after MI, functions distinct from effects on the atherosclerotic plaques. Our experiments, performed with bone marrow chimeras, demonstrate an effect on MI healing with a  $\approx 80\%$  knockdown of Lp-PLA<sub>2</sub>, indicating this is a sufficient reduction to elicit positive effects. Future studies will need to elucidate in more detail the full scope of Lp-PLA<sub>2</sub> function not only on leukocyte behavior but also on effects attributed to other cells in the heart, such as fibroblasts and endothelial cells.

Recently, 2 large multicenter phase III trials completed evaluation of the Lp-PLA<sub>2</sub> inhibitor darapladib on the reduction of cardiovascular adverse events in >28 000 patients with

documented coronary heart disease. These 2 complementary trials covered both chronic and acute coronary heart disease and evaluated traditional coronary heart disease end points. Thus, these studies did not formally focus on LV function or long-term development of heart failure.<sup>25,26</sup> The Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial enrolled patients within 30 days of acute coronary syndrome and the STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY (STABILITY) trial enrolled patients with stable chronic coronary heart disease. Despite promising preclinical results,<sup>10</sup> both clinical trials concluded that direct inhibition of Lp-PLA<sub>2</sub> with darapladib failed to reduce major adverse cardiovascular events.<sup>12,27</sup> Crucially, the trials did not test whether blockade of Lp-PLA<sub>2</sub> per se was beneficial: patients receiving darapladib or placebo were already receiving  $\leq 4$  different therapeutics against heart disease, including statins and ACE inhibitors. The trials, therefore, showed that under the specific parameters of the study, darapladib did not provide benefit above that afforded by current treatment regimens.

Our results provide an alternative explanation as to why the clinical trials might not have improved the tested end points. Our data show that, as early as day 1 after MI, expression of Lp-PLA<sub>2</sub> was increased. In the absence of Lp-PLA<sub>2</sub>, inflammatory leukocyte recruitment was blunted, yielding a smaller inflammatory response that correlated with improved healing and heart function 7 and 21 days later, respectively. Lp-PLA<sub>2</sub> might elicit its most detrimental effects in the acute phase after MI. If so, blocking the enzyme earlier than the SOLID or STABILITY trials might benefit end points related to LV function. Several human studies have shown data in support of the idea that healing of the infarcted myocardium involves the biphasic accumulation of monocytes and macrophages. In 1 study, a cohort of 36 patients monitored >2 weeks after MI demonstrated a peak of circulating inflammatory CD16<sup>-</sup> monocytes on day 2.6 after MI, followed by another peak of CD16<sup>+</sup> monocytes on day 4.8.<sup>19</sup> Because CD16<sup>-</sup> monocytes resemble inflammatory Ly-6C<sup>high</sup> monocytes, whereas CD16<sup>+</sup> monocytes (and in particular CD16<sup>+</sup> CD14<sup>dim</sup>) resemble Ly-6C<sup>low</sup> monocytes, these findings suggest that acute inflammation may likewise peak in humans within 1 week after MI. Immediate inhibition of Lp-PLA<sub>2</sub> might be the optimal time window for improving outcomes related to LV function. The SOLID trial, which enrolled patients within 30 days, did not test whether patients receiving darapladib within the first 3 days post MI benefited from treatment. Hence, the clinical trial findings do not argue against the role of Lp-PLA<sub>2</sub> in inflammation after MI.

In summary, the data demonstrate that Lp-PLA<sub>2</sub> regulates the host response after MI through modulation of inflammation. By enhancing inflammation and impairing repair, the induced Lp-PLA<sub>2</sub> negatively regulates recovery of LV function. In addition to stimulating atherosclerotic plaque vulnerability, the effect of Lp-PLA<sub>2</sub> after MI reveals a novel role of this enzyme in modulating the myocardial response to ischemic injury. These mechanistic insights have implications for developing effective therapeutics against ischemic cardiomyopathy and chronic heart failure post MI.



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## Disclosures

None.

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### CLINICAL PERSPECTIVE

Myocardial infarction involves balanced accumulation of monocytes and macrophages in tissue. This study shows that lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) is involved in the regulation of host response after myocardial infarction through modulating inflammatory responses. Elevated Lp-PLA<sub>2</sub> after myocardial infarction associated with heightened tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$  and IL-6 levels in serum. Accordingly, increased numbers of neutrophils and inflammatory Ly-6C<sup>high</sup> monocytes accumulated into the infarcted myocardium. Depletion of Lp-PLA<sub>2</sub> gave rise to Ly-6C<sup>low</sup> macrophages with a skewed M2-prone gene expression signature in the reparative phase, leading to increased collagen deposition, fewer inflammatory cells, and improved indices of angiogenesis. Consequently, the hearts of *bmLp-PLA<sub>2</sub><sup>-/-</sup>* mice healed more efficiently, as observed by improved left ventricle remodeling and ejection fraction. Lp-PLA<sub>2</sub> thus may serve as a therapeutic target for heart failure after myocardial infarction.