Lp-PLA₂ 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-PLA₂), a member of the phospholipase A<sub>2</sub> family of enzymes, produced predominantly by leukocytes, participates in host defenses and disease. Elevated Lp-PLA₂ 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-PLA₂ in healing after MI.

Methods and Results—In response to MI, Lp-PLA₂ levels markedly increased in the circulation. To test the functional importance of Lp-PLA₂, we generated chimeric mice whose bone marrow–derived leukocytes were Lp-PLA₂−/− deficient (bmLp-PLA₂<sup>−/−</sup>). Compared with wild-type controls, bmLp-PLA₂<sup>−/−</sup> mice subjected to MI had lower serum levels of inflammatory cytokines tumor necrosis factor-α, interleukin (IL)-1β, and IL-6, and decreased number of circulating inflammatory myeloid cells. Accordingly, bmLp-PLA₂<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-PLA₂<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-PLA₂<sup>−/−</sup> mice healed more efficiently, as determined by improved left ventricle remodeling and ejection fraction.

Conclusions—Lp-PLA₂ 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. 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.

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

Lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}), a member of the phospholipase A\textsubscript{2} family of enzymes, hydrolyses glycerophospholipids. The ensuing enzymatic reactions frequently generate metabolic signaling molecules with a multitude of biological actions. For example, by hydrolyzing phosphotidylcholine at C2 of the glycerol backbone, Lp-PLA\textsubscript{2} produces lyso phosphatidylcholine,\textsuperscript{7} which fosters oxidative stress, affects vascular smooth muscle cell proliferation, and increases tissue accumulation of macrophages.\textsuperscript{6} Several observational studies showed that Lp-PLA\textsubscript{2} levels correspond to MI, \textit{and} sudden cardiac death.\textsuperscript{7-9} Although preliminary studies reported reduced development of advanced coronary atherosclerosis\textsuperscript{10} or stabilization of the necrotic core size\textsuperscript{11} with selective inhibiting Lp-PLA\textsubscript{2}, in cardiovascular disease.\textsuperscript{12} Neither study focused on LV function nor chronic heart failure end points. Moreover, it remains unclear whether Lp-PLA\textsubscript{2} 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\textsubscript{2} participates in infract healing and HF after MI.

\textbf{Methods}

Further details are available in the Data Supplement.

\textbf{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\textsubscript{2}−/− mice 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\textsubscript{2}−/− bone marrow to generate respective chimeric mice. The chimeras had normal leukocyte counts and exhibited no obvious abnormalities, consistent with the Lp-PLA\textsubscript{2}−/−-deficient mice.\textsuperscript{13} MI was induced by permanent ligation of the left anterior descending artery. We observed no differences in mortality between the groups.

\textbf{Lp-PLA\textsubscript{2}/PAF Acetyl-Hydrolase Activity Assay}

The PAF hydrolase activity assay was performed as previously described\textsuperscript{14} with modifications using [\textsuperscript{1}H]PAF (Platelet Activating Factor, 1-O-Hexadecyl-[Acetyl-\textsuperscript{1}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-\textsuperscript{1}H]-, Hexadecyl PAF, [acetyl-\textsuperscript{1}H]-, (250μCi (9.25MBq) was purchased from Perkin Elmer, and Bio-Safe II was purchased from Research Products International Corp, Mount Prospect, IL.

\textbf{Histology}

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

\textbf{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.

\textbf{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).

\textbf{Magnetic Resonance Imaging}

Magnetic resonance imaging was performed on days 1 and 21 after permanent coronary ligation as described previously.\textsuperscript{15} 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.\textsuperscript{15} 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.

\textbf{Statistics}

Results are shown as means±SEM. Unpaired Student \textit{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. \textit{P} values ≤0.05 denote significant changes.

\textbf{Results}

\textbf{Expression of Lp-PLA\textsubscript{2} After MI and Its Role on Healing}

To elucidate whether Lp-PLA\textsubscript{2} participates in healing after MI, we first measured Lp-PLA\textsubscript{2} expression and serum activity in steady state and after MI. Lp-PLA\textsubscript{2} 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\textsubscript{2} may participate in myocardial ischemic injury. Concordantly, Lp-PLA\textsubscript{2} activity was also increased shortly after MI (Figure 1B). To define the role of Lp-PLA\textsubscript{2} in hemato poetic 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\textsubscript{2}−/− (bmLp-PLA\textsubscript{2}−/−) mice. In comparison with WT mice, bmLp-PLA\textsubscript{2}−/− mice had lower Lp-PLA\textsubscript{2} activity at steady state, and this activity did not change after MI (Figure 1B). These findings establish leukocytes as major sources of Lp-PLA\textsubscript{2} in response to MI. We then evaluated infract healing and demonstrated a significant decrease in infract size in bmLp-PLA\textsubscript{2}−/− mice 7 days after MI compared with WT (Figure 1C and 1D).
Lp-PLA₂ Influences Systemic Inflammation

Coronary occlusion stimulates an inflammatory response characterized by cytokine and chemokine production, and leukocyte recruitment to the heart. Because Lp-PLA₂ participates in inflammation, we assessed the effect of Lp-PLA₂ deficiency after MI. Serum concentrations of inflammatory cytokines tumor necrosis factor-α, IL-1β, 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-PLA₂−/− mice showed only moderately elevated tumor necrosis factor-α and IL-6, and negligibly increased IL-1β, demonstrating a diminished inflammatory response in the absence of Lp-PLA₂ (Figure 2A). Time-course profiling of circulating leukocytes after MI revealed that both WT and bmLp-PLA₂−/− mice augmented inflammatory myeloid cells (neutrophils and Ly-6C high monocytes), but compared with WT mice, bmLp-PLA₂−/− mice had fewer neutrophils and Ly-6C high cells in blood at days 3 and 7, indicating Lp-PLA₂ contributes to the systemic inflammation after MI (Figure 2B and 2C).
Lp-PLA₂ Impairs the Appearance of Reparative Macrophages

The myocardium displays a biphasic monocyte and macrophage response during MI. 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-PLA₂<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. Yet, for nearly every peak, infarcts of bmLp-PLA₂<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-PLA₂ 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-PLA₂ shapes macrophage function by measuring expression of signature M1/M2 genes in sorted cardiac macrophages. In comparison with WT macrophages, Lp-PLA₂<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 lyso-phosphotidylcholine, a product of Lp-PLA₂, potentiates an M1-like macrophage phenotype. Together, these data show that Lp-PLA₂ promotes recruitment of inflammatory myeloid cells and delays the appearance of reparative macrophages in the ischemic myocardium.

Lp-PLA₂ Retards Healing After MI

The differences in leukocyte recruitment between WT and bmLp-PLA₂<sup>−/−</sup> mice prompted us to determine whether the absence of Lp-PLA₂ 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-PLA₂<sup>−/−</sup> mice accumulated fewer myeloid CD11b<sup>+</sup> cells, indicating less severe inflammation. Infarcts of bmLp-PLA₂<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<sup>+</sup>area). Collectively, the results of histological examination demonstrate more effective healing in the absence of Lp-PLA₂. These results demonstrate that Lp-PLA₂ inhibits the resolution of inflammation after MI.

Improved Heart Function in the Absence of Lp-PLA₂

To test whether inflammation mediated by Lp-PLA₂ after MI translated to impaired heart function, we performed magnetic resonance imaging in vivo in WT and bmLp-PLA₂<sup>−/−</sup> mice. In the steady state, we detected no differences in cardiac function

![Figure 3. Attenuated inflammatory response in bmLp-PLA₂<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-PLA₂<sup>−/−</sup> mice. B, Flow cytometry based quantification of neutrophil, monocyte and MΦ (macrophage) numbers in MI tissue of WT versus bmLp-PLA₂<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-PLA₂<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-PLA₂<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.](image-url)
between WT and bmLP-PLA \(_{-/-}\) 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\(_2\). Moreover, compared with day 1, ejection fraction at day 21 diminished in WT mice but increased modestly in bmLP-PLA \(_{-/-}\) mice, suggesting improved recovery of heart function in the absence of Lp-PLA\(_2\). Overall, the data show that Lp-PLA\(_2\) aggravates LV remodeling and impairs LV function after MI.

**Discussion**

Recruitment of neutrophils and Ly-6Chigh monocytes into the infarcted myocardium and the subsequent generation of reparative macrophages from Ly-6Chigh 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.\(^{17-19}\) This study shows that deficiency of Lp-PLA\(_2\) on hematopoietic cells attenuates systemic inflammation after MI, impairs leukocyte infiltration into infarcts, and enhances generation of reparative Ly-6C\(^{low}\) macrophages, leading to less adverse LV remodeling and improved recovery of LV function. Together, the data show that Lp-PLA\(_2\) modulates inflammation after MI and suggest that targeting of Lp-PLA\(_2\) might lessen LV dysfunction and the development of chronic heart failure after MI.

Monocyte-derived macrophages can produce substantial Lp-PLA\(_2\),\(^{20}\) and in the mouse, Lp-PLA\(_2\) is expressed almost exclusively by myeloid cells (www.immgen.org). Previous studies have identified macrophage Lp-PLA\(_2\) expression at both the mRNA and the protein levels in human and rabbit aortic lesions.\(^{21}\) Plaques with characteristics of vulnerable and ruptured atheromata, but not early lesions, contain abundant Lp-PLA\(_2\).\(^{22}\) Therefore, extensive efforts have been taken to prevent atherosclerosis-related coronary heart disease by inhibiting Lp-PLA\(_2\) and thus promoting plaque stability.\(^{15}\) But Lp-PLA\(_2\) may participate in coronary heart disease by modulating inflammation independent of effects on the plaque itself. For example, our data showed that Lp-PLA\(_2\) rose dramatically during MI. As a phospholipase, increased Lp-PLA\(_2\) hydrolyzes phospholipids of oxidatively damaged cells or lipoproteins. Lp-PLA2 hydrolyzes oxidized phosphatidylcholine,
with this hypothesis, we found that bmLp-PLA2 volume; EF, ejection fraction; and LVM, left ventricle volume. * respectively. 

detail the full scope of Lp-PLA2 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-PLA2 inhibitor darapladib on the reduction of cardiovascular adverse events in >28000 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.25,26 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,10 both clinical trials concluded that direct inhibition of Lp-PLA2 with darapladib failed to reduce major adverse cardiovascular events.12,27 Crucially, the trials did not test whether blockade of Lp-PLA2 per se was beneficial: patients receiving darapladib or placebo were already receiving ≤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-PLA2 was increased. In the absence of Lp-PLA2, 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-PLA2 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+ monocytes on day 2.6 after MI, followed by another peak of CD16+ monocytes on day 4.8.19 Because CD16+ monocytes resemble inflammatory Ly-6Cdim monocytes, whereas CD16+ monocytes (and in particular CD16+CD14dim) resemble Ly-6Cdim monocytes, these findings suggest that acute inflammation may likewise peak in humans within 1 week after MI. Immediate inhibition of Lp-PLA2 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-PLA2 in inflammation after MI.

In summary, the data demonstrate that Lp-PLA2 regulates the host response after MI through modulation of inflammation. By enhancing inflammation and impairing repair, the induced Lp-PLA2 negatively regulates recovery of LV function. In addition to stimulating atherosclerotic plaque vulnerability, the effect of Lp-PLA2, 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.
Acknowledgments

We thank Michael Waring and Nathalie Bonheur for sorting cells. We also thank Dr Partha Datta for assistance with mouse irradiation.

Sources of Funding

This work was supported, in part, by GSK funding (GlaxoSmithKline: Targeting Lp-PLA2, in Inflammatory Cardiovascular Disease) and by National Institutes of Health grants 1R01HL095612 and R56AI104695 (to Dr Swirski).

Disclosures

None.

References


Myocardial infarction involves balanced accumulation of monocytes and macrophages in tissue. This study shows that lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is involved in the regulation of host response after myocardial infarction through modulating inflammatory responses. Elevated Lp-PLA₂ after myocardial infarction associated with heightened tumor necrosis factor-α, interleukin (IL)-1β and IL-6 levels in serum. Accordingly, increased numbers of neutrophils and inflammatory Ly-6Chigh monocytes accumulated into the infarcted myocardium. Depletion of Lp-PLA₂ gave rise to Ly-6Clo≈ 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-PLA₂⁻/⁻ mice healed more efficiently, as observed by improved left ventricle remodeling and ejection fraction. Lp-PLA₂ thus may serve as a therapeutic target for heart failure after myocardial infarction.