ORIGINAL RESEARCH

Inflammation, Autophagy, and Apoptosis After Myocardial Infarction

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***Background*—**There is evidence for inflammation, autophagy, and apoptosis in the ischemic heart. Autophagy is a physiologic process for tissue survival. Apoptosis, on the other hand, is a mechanism that serves to clear the debris in the setting of tissue injury. The balance between autophagy and apoptosis may be important in cell survival and cardiac function.

***Methods and Results*—**We examined the interplay of inflammation and myocyte autophagy and apoptosis during the ischemic process. We subjected mice to total left coronary artery ligation and studied these animals for up to 4 weeks. The inflammatory (tumor necrosis factor [TNF]-α, monocyte chemoattractant protein-1, interleukin-6, and interleukin-1β) and autophagic signals (light chain-3 and beclin-1) were strongest during the first week and then began to decline. However, the apoptotic signals peaked at week 2 after left coronary artery ligation, and the elevated levels persisted until the end of the fourth week. To elucidate the role of inflammation in the regulation of myocyte autophagy and apoptosis, we administered TNF-α inhibitor (CAS1049741-03-8, Millipore, Burlington, MA) to the mice daily during the first week of myocardial infarction. Anti-TNF-α therapy reduced the levels of inflammatory cytokines and the inflammatory cell infiltration in and around the infarct area. However, cardiac function measured by echocardiography (fractional shortening and ejection fraction) worsened with anti-TNF-α therapy. More importantly, application of TNF-α inhibitor markedly inhibited autophagy and promoted myocyte apoptosis in the border zone.

***Conclusions*—**These observations suggest that inflammatory response may be protective in the early stage of the myocardial infarction through stimulation of myocyte autophagy. Anti-inflammatory treatment early after coronary occlusion may have an adverse effect. **(*J Am Heart Assoc*. 2018;7:e008024. DOI: 10.1161/JAHA.117.008024.)**

Key Words: apoptosis • autophagy • inflammation • myocardial infarction

It is generally believed that there is intense systemic and local inflammation after myocardial infarction (MI).[1] Degree of inflammation is viewed as a major determinant of extent of MI and subsequent cardiac remodeling and function. Several inflammatory markers, such as C-reactive protein and interleukin (IL)-6 are elevated in the post-MI state.[2,3] Based on these observations, several trials of anti-inflammatory drugs in patients with acute myocardial ischemia are currently underway.[3,4]

It has been proposed that inflammation plays different roles in the development of cardiac remodeling following onset of myocardial ischemia, and the population of inflammatory cells changes dynamically with the progression of myocardial ischemia.[5,6] The dynamic changes in leukocyte subsets in the ischemic myocardium are time-dependent and site specific. These alterations in inflammatory patterns may have protective or detrimental effects on cardiac function during chronic ischemia.[5,6]

Autophagy is a major cellular process that is involved in the degradation and recycling of the unnecessary and dysfunctional proteins, cellular organelles, and other cellular components.[7] Apoptosis is a process of programmed cell death that is a naturally occurring homeostatic process in the body that regulates cell death. Autophagy has complex relationships with apoptosis. The balance between autophagy and apoptosis determines cell survival.

A previous study from our laboratory showed that cardiomyocytes develop autophagy early when exposed to hypoxia, whereas apoptosis occurs late and persists for a long time on exposure of cells to hypoxia. Similarly cardiomyocytes in culture, when exposed to angiotensin II (Ang II), a peptide

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An accompanying Figure S1 is available at http://jaha.ahajournals.org/content/7/9/e008024/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- Left coronary artery ligation in mice leads to early inflammation and autophagy and late apoptosis in and around the infarct area.
- Anti–tumor necrosis factor-α therapy suppresses inflammation and autophagy, whereas apoptosis increases in association with a decrease in cardiac function.

What Are the Clinical Implications?

- Inflammation may be protective in the early stage of the myocardial infarction through stimulation of myocyte autophagy.
- Potent anti-inflammatory treatment after coronary occlusion in the early stage may have an adverse effect, perhaps by inhibiting autophagy.

released in the ischemic heart, develop autophagy early and in response to small concentrations of Ang II; in contrast, cells develop apoptosis when exposed to high concentrations of Ang II.[7] Ding et al observed a similar dynamic alteration in autophagy and apoptosis in vascular smooth muscle cells exposed to oxidized low-density lipoprotein.[8] These observations suggest that the cell protective mechanisms are operative soon after ischemia, and the cell injury signals occur late during sustained hypoxia or on exposure of cells to Ang II or other oxidant stimuli.

The present study was designed to investigate the interplay of inflammation and development of autophagy and apoptosis in the post-MI state in a mouse model. We also studied the impact of inhibition of inflammatory signals on the evolution of infarct size and cardiac function and appearance of autophagy and apoptosis.

Materials and Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. Other researchers can contact the corresponding authors about methodological questions.

Left Coronary Artery Ligation Protocol

Adult male C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The animal study protocol was approved by the local institutional animal use care committee and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Academies Press (Washington, DC). Intense myocardial ischemia was induced by total ligation of left coronary artery (LCA) in the anesthetized mice as recently published protocols.[9] The animals were euthanatized at the end of 1, 2, 3, or 4 weeks after LAC ligation. In the second part of the study, a group of mice were given a selective tumor necrosis factor (TNF)-α inhibitor (CAS1049741-03-8, Millipore) dissolved in saline (25 mg/kg per day) by intraperitoneal injection for 7 days. Control mice were injected with saline and handled in a similar fashion.

Echocardiographic Assessment

The echocardiographic assessment was performed as recently described.[9] The B-mode and M-mode echocardiography were recorded with a 707 ultrasound system (VisualSonics Inc, Toronto, ON, Canada) following 1 to 4 weeks of ischemia, and fractional shortening was calculated.

Assessment of Infarct Size

Following euthanasia of the animals, mouse hearts were collected, weighed, and rinsed with cooled PBS, and their pictures were taken immediately at 4°C. The whole hearts were frozen at −20°C and subsequently sliced into 1-mm-thick sections from the apex to the base. The slices were incubated in 1% buffered triphenyltetrazolium chloride (Sigma-Aldrich, St. Louis, MO) at 37°C for 15 minutes to stain the viable myocardium brick red. Each slice was imaged with computer-assisted planimetry (NIH Image J 1.34s), and the infarct size was then assessed. Six hearts in each group were used to assess infarct size.

Hematoxylin and Eosin Staining

Left ventricular (LV) sections (6 samples/group) were deparaffinized, rehydrated, and stained with hematoxylin and eosin per standard protocols. The images were captured by a digital imaging system.

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling

LV sections were treated per the above-mentioned protocols. Apoptosis was detected using a DeadEnd™ Fluorometric TUNEL kit (Promega Corporation, Madison, WI) per manufacturer's instructions.

Immunostaining

LV sections (6 samples/group) were deparaffinized in xylene and rehydrated through gradient ethanol (100% to 70%), permeabilized by 1% Triton X-100, and immersed in 3% H_2O_2 for 10 minutes to inactivate endogenous peroxidase activity. The sections were blocked with 5% goat serum/1% BSA in

PBS for 30 minutes at room temperature and then incubated with anti-LC3-A/B antibody (1:400, v/v, dilution, Cell Signaling Technology, Danvers, MA) or anti-CD68 antibody (1:400, v/v, dilution, Abcam, Cambridge, UK) in 3% goat serum/1% BSA at 4°C overnight. After washing with PBS 3 times, the sections were incubated with FITC-conjugated (Abcam) secondary antibody (1:1000, v/v, dilution) in 3% goat serum/1% BSA for 30 minutes at room temperature. After being washed with PBS and deionized water, the sections were covered with coverslips by ProlongH Gold antifade reagent with 4,-diamidino-2-phenylindole (DAPI; Life Technologies, Carlsbad, CA).

Quantitative Reverse-Transcriptase Polymerase Chain Reaction

Total RNA was isolated from LV samples using RNeasy Mini-Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Before use, RNA was treated with DNase I and diluted to 100 μg/mL. One microgram of RNA was reverse-transcribed into cDNA with SuperScript II 1st Strand DNA Synthesis Kit (Invitrogen, Carlsbad, CA). Quantitative polymerase chain reaction (qPCR) was performed using the Applied Biosystems Fast 7500HT real-time PCR system with a 15-μL reaction volume including 7.5 μL GoTaq qPCR Master Mix (Promega, Madison, WI), 100 ng of cDNA, and 0.3 μmol/L primers. Data analysis was performed using MX3000P software. Relative mRNA expression was quantified using the comparative threshold cycle method. The sequences of primers used for PCR assay are shown as follows: TNF-α, F-5′-ACCCTCACACTCAGATCATCTTC-3′, R-5′-TGGTGGTTTGCTACGACGT-3′; monocyte chemoattractant protein [MCP]-1, F-5′-CCACTCACCTGCTGCTACTCA-3′, R-5′-TGGTGATCCTCTTGTAGCTCTCC-3′; IL-6, F-5′-GTTGCCTTCTTGGGACTGATG-3′, R-5′-TGGGAGTGGTATCCTCTGTGAA-3′; IL-1β, F-5′-TTCAAATCTCGCAGCAGCAC-3′, R-5′-GTGCAGTTGTCTAATGGGAACG-3′; GADPH, F-5′-AGGTCGGTGTGAACGGATTTG-3′, R-5′-TGTAGACCATGTAGTTGAGGTCA-3′.

Western Blotting

Proteins were extracted from LV samples and/or border zones (5 samples/group) using radioimmunoprecipitation assay lysis buffer (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) supplemented with protease inhibitor, phosphatase inhibitor, and phenylmethylsulfonyl fluoride. The protein concentration was measured using the Bradford assay. Proteins (20 μg/sample) were diluted in an equal volume of 2× Laemmli sample buffer (Bio-Rad, Hercules, CA) with 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), heated at 95°C for 5 minutes, loaded into 12% precast SDS/PAGE gels (Bio-Rad, Hercules, CA), and separated by electrophoresis. Following electrophoresis, proteins were transferred onto nitrocellulose blotting membranes (GE Healthcare Life Science, Marlborough, MA). The membranes were blocked by 5% nonfat milk in TBS with Tween-20 for 1 hour at room temperature, and then incubated with primary antibodies LC3A/B (Cell Signaling Technology, Danvers, MA), Beclin-1 and cleaved caspase-3 (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) at 4°C overnight. After washing 3 times with TBS with Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:10 000, v/v, dilution) for 1 hour at room temperature. The immunoreactive bands were visualized after incubation with Western Blotting Luminol Reagents (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) for 3 minutes and then exposed to Blue Basic Autorad Film (Andwin Scientific, Woodland Hills, CA). The protein bands were scanned and quantified using Image J software.

Enzyme-Linked Immunosorbent Assay

Total proteins were extracted from the LV tissues of the model mice (8 animals/group). The levels of IL-6 and TNF-α in the protein solution were measured using mouse IL-6 ELISA Ready-SET-Go kit and mouse TNF-α ELISA Ready-SET-Go kit (eBioscience, Inc, San Diego, CA), respectively, per the manufacturer's instructions. The concentrations of both cytokines were calculated from the standard curve.

Statistical Analysis

Statistical analysis was performed using SPSS 15.0 software (IBM, Chicago, IL). Data are presented as means±SDs from 5 to 8 independent experiments. Univariate comparisons of means were evaluated using 1-way ANOVA with Tukey post hoc adjustment for multiple comparisons. A $P<0.05$ was considered statistically significant.

Results

Infarct Size and Cardiac Function

Cardiac function was measured in all animals at the end of 1, 2, 3, and 4 weeks after LCA ligation. The animals were then euthanatized and the infarct size was measured. As shown in Figure 1A, heart weight increased following LCA ligation, was maximum at 1 week ($P<0.05$ versus sham ischemia mice), and then decreased in a time-dependent manner ($P<0.05$). This indicates myocardial hypertrophy in the early stage of myocardial infarction. The LV then begins to lose its thickness (or begins to dilate). This was confirmed by triphenyltetrazolium chloride staining of representative sections of the heart (Figure 1A, bottom).

Data on LV diameters are shown in Figure 1B. The systolic and diastolic LV dimensions increased over time (1-4 weeks

after LCA ligation). Importantly, both fractional shortening and ejection fraction declined progressively over the 4-week period following LCA ligation.

Apoptosis Signals After LCA Ligation

We measured occurrence of apoptosis by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining (immunofluorescence) and cleaved caspase-3 (Western blotting) in the LV tissues post-LCA ligation. As shown in Figure 2A, both positivity of TUNEL staining and expression of cleaved caspase-3 increased at 1 week and continued to increase, reaching maximal level at 2 weeks after LCA ligation and then stabilizing at high levels. Of note, apoptosis was present mostly in the zone bordering the infarct area as well as in the infarct area.

Autophagy Signals After LCA Ligation

We measured LC3 (immunostaining and Western blotting) as a marker of autophagy in the LV tissues following LCA ligation. As another marker of autophagy, we measured beclin-1 levels (Western blotting). As shown in Figure 2B, expression of both LC3 and beclin-1 increased severalfold at 1 week after LCA ligation (versus sham ischemia mice) and then gradually declined over the subsequent 3-week period. The initial increase and subsequent decline in all parameters of autophagy were generally similar. The results of LC3 and beclin-1 expression were similar. Of note, LC3 immunostaining was present mostly in the zone bordering the infarct area.

Inflammatory State After LCA Ligation

We measured a host of inflammatory signals (TNF-α, MCP-1, IL-6, and IL-1β) by qPCR in the LV tissues after LCA ligation. As shown in Figure 3A, TNF-α, MCP-1, IL-6, and IL-1β mRNA levels increased severalfold at 1 week after LCA ligation (versus sham ischemia mice) and then gradually declined over the subsequent 3-week period. The initial increase and subsequent decline were generally similar for all cytokines. We also measured TNF-α and IL-6 levels in the LV tissues by ELISA and observed that the protein levels followed the mRNA levels (Figure 3B).

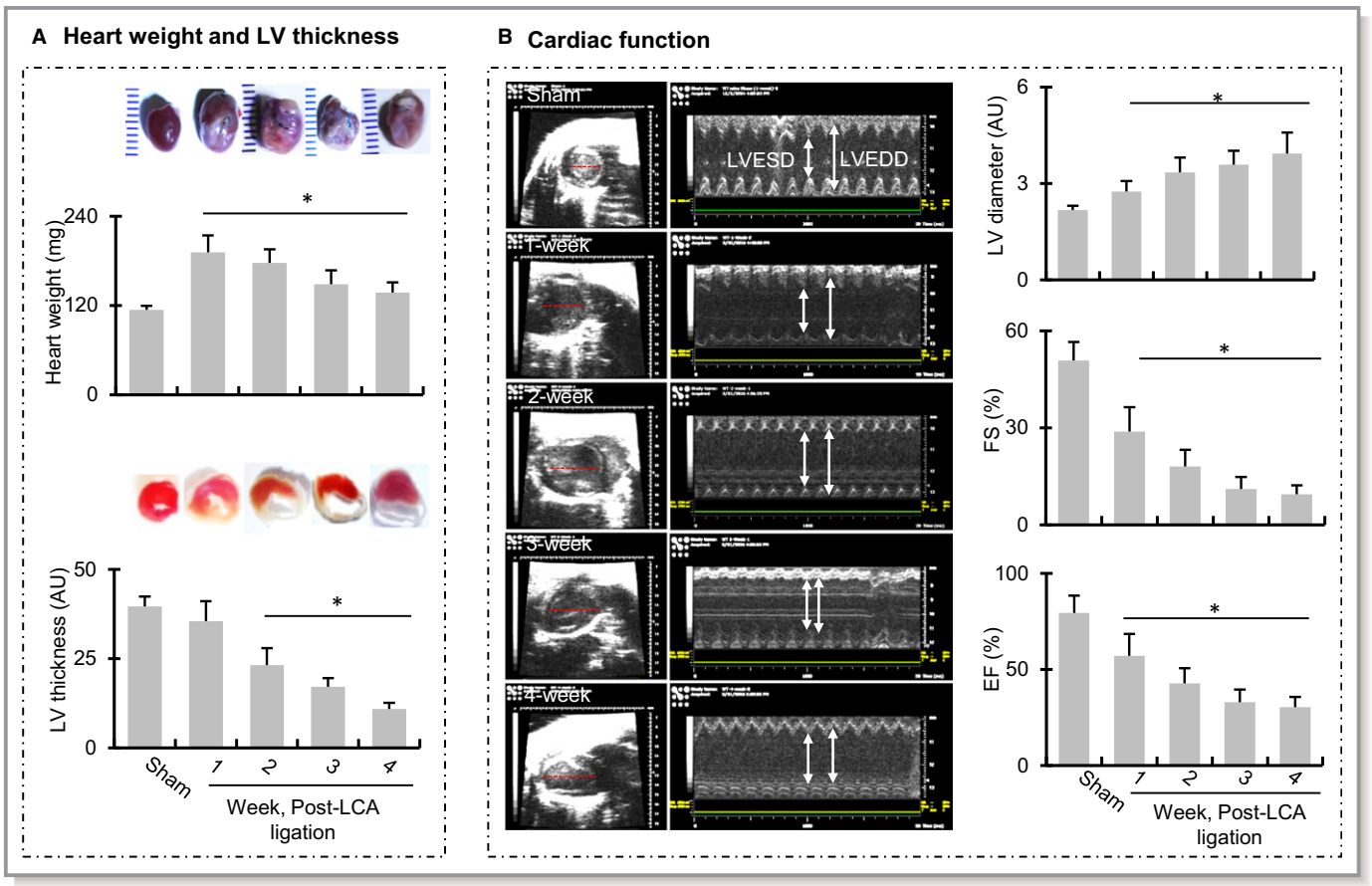

Figure 1. Left coronary artery ligation and cardiac function at 1, 2, 3, and 4 weeks following LCA ligation. A, Heart weight and left ventricular thickness. B, Representative B-mode and M-mode echocardiographic images and quantification of LV diameter, fractional shortening, and ejection fraction at different time-points. *$P<0.05$ vs sham group. EF indicates ejection fraction; FS, fractional shortening; LCA, left coronary artery; LV, left ventricle; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension.

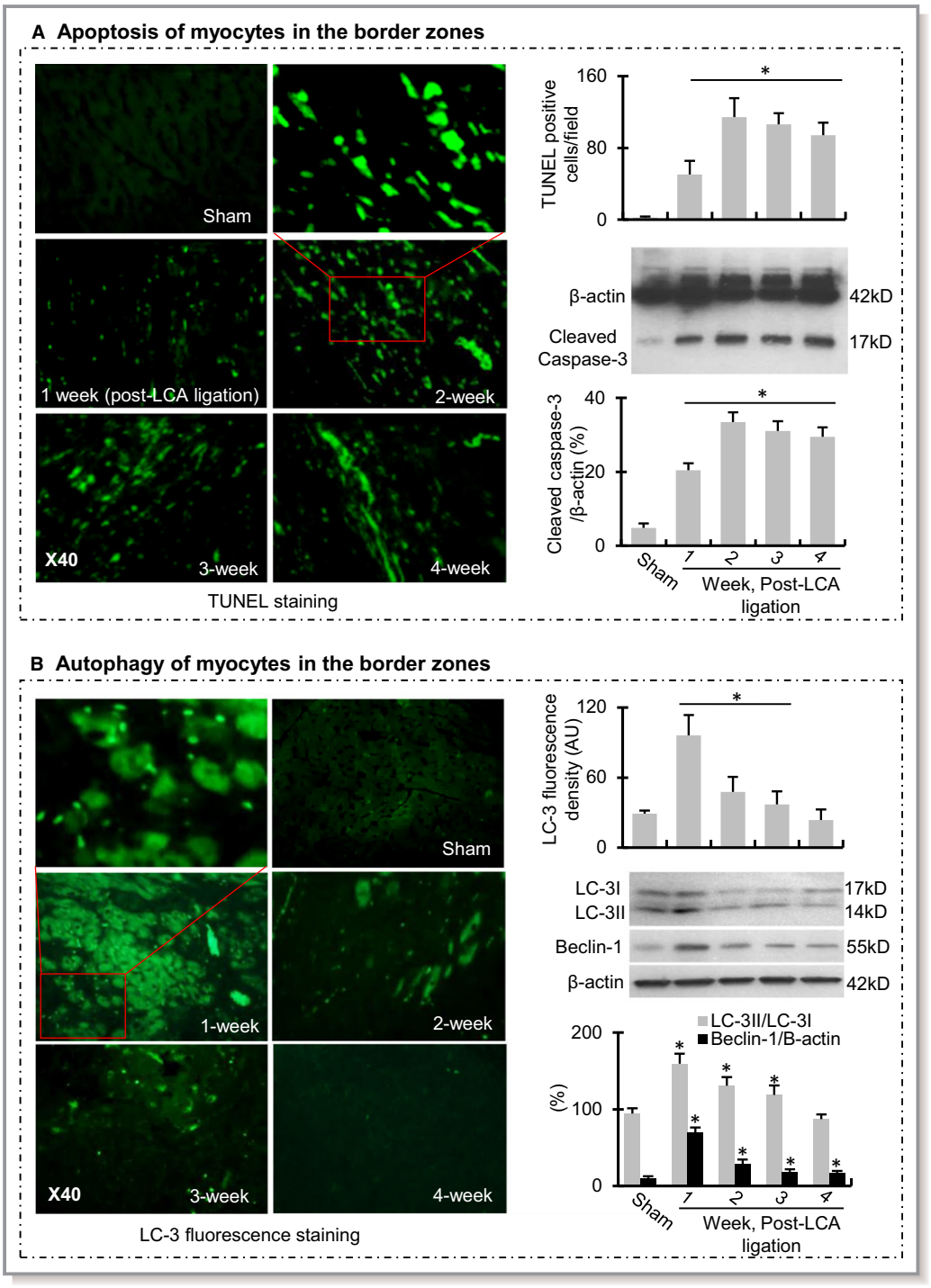

Figure 2. Apoptosis and autophagy in the border zones of infarct area at 1, 2, 3, and 4 weeks following LCA ligation (amplification ×4). A, Apoptosis in the border zones determined by TUNEL staining and cleaved-caspase-3 expression by Western blotting assay. B, Autophagy in the border zones indicated by LC-3 fluorescence staining, the ratio of LC-3II vs LC-3I and beclin-1 expression by Western blotting. **P*<0.05 vs sham group. LCA indicates left coronary artery; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

Relationship Among Inflammation, Autophagy, and Apoptosis After LCA Ligation

As shown in Figure S1A, the changes in inflammation marker TNF-α and beclin-1 were parallel, suggesting that inflammation may be related to the development of autophagy. On the other hand, as shown in Figure S1B, the expression of caspase-3 paralleled the increase in TNF-α in the early stages of ischemia; thereafter, TNF-α level declined

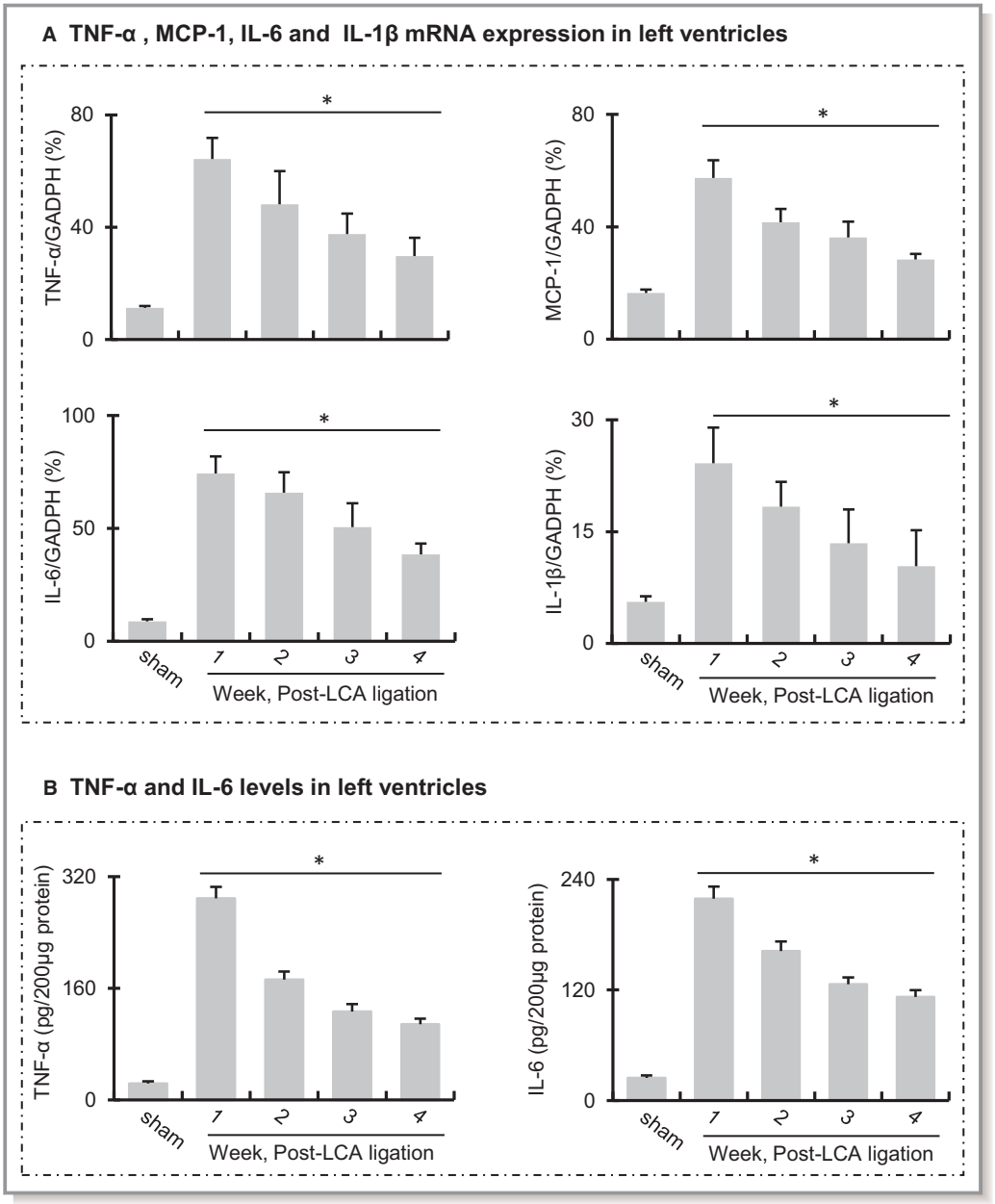

Figure 3. Inflammatory response in the cardiac tissue at 1, 2, 3, and 4 weeks following LCA ligation. A, TNF-α, MCP-1, IL-6, and IL-1β mRNA (qPCR) in the LV tissues. B, TNF-α and IL-6 protein levels (ELISA) in the LV tissues. **P*<0.05 vs sham group. LCA indicates left coronary artery; LV, left ventricle; qPCR, quantitative polymerase chain reaction.

significantly, but the increase in caspase-3 was persistent. The dissociation between the appearance of autophagy and apoptosis also became evident in the post-LCA ligation period when autophagy signals began to fall although apoptosis signals continued to increase (Figure S1C).

Causative Links Among Inflammation, Autophagy, and Apoptosis

We determined the causative links among inflammation, autophagy, and apoptosis by treating groups of mice with saline solution or a potent TNF-α inhibitor daily during the first week in the post–LCA ligation period. The infarct size at 1 week post–LCA ligation varied widely, but overall there was no change in infarct size in the saline- and TNF-α inhibitor–treated mice. The efficacy of the anti-inflammatory effect of the TNF-α inhibitor became evident from a marked inhibition of leukocyte accumulation and a significant reduction in CD68 expression (immunofluorescence) and IL-6 expression (immunostaining) (Figure 4A through 4F). Application of TNF-α inhibitor also markedly inhibited mRNA expression of TNF-α, IL-6, and MCP-1, indicating the potency of the inhibitor (Figure 4G through 4I).

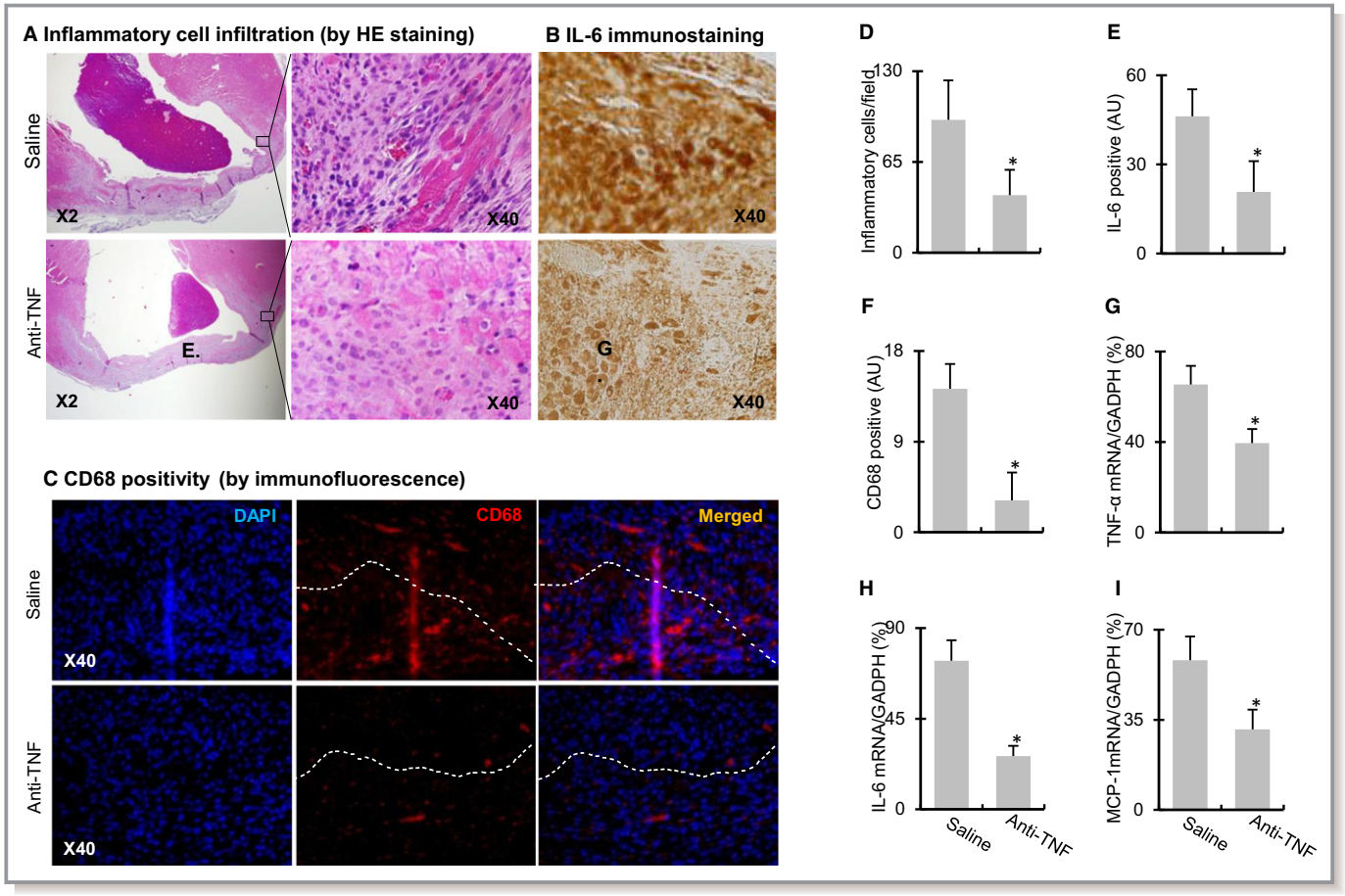

Figure 4. Inflammatory response in the heart at 1 week following LCA ligation. Mice treated with saline or TNF-α inhibitor. A, Representative hematoxylin and eosin (HE) staining showing infarct areas and inflammatory cell infiltration (amplification ×2 and ×40). B, IL-6 immunochemistry staining (amplification ×40). C, CD68 immunofluorescence staining (amplification ×40). D through I, Quantification of inflammation cell infiltration, IL-6 and CD68 staining, and TNF-α, IL-6, and MCP-1 mRNA expression. *P<0.05 vs the mice given saline solution. LCA indicates left coronary artery.

We also examined the effect of anti–TNF-α therapy on cardiac function. There appeared to be a significant reduction in fractional shortening and ejection fraction in mice treated with the TNF-α inhibitor compared with those treated with saline (P<0.05) (Figure 5).

We also examined apoptosis in the hearts of mice treated with saline or the TNF-α inhibitor and subjected to LCA ligation. As shown in Figure 6A, 6C, and 6D, the difference of apoptotic positivity (fluorescence TUNEL staining) between the TNF-α inhibitor–treated mice and saline-treated mice was only seen in the area bordering the infarct. The number of apoptotic cells (TUNEL positivity) increased by almost 50% in the border zone of mice treated with TNF-α inhibitor (P<0.05 versus saline-treated mice). However, there was no significant difference in the number of apoptotic cells in the infarct zone between the 2 groups of mice (Figure 6C).

Next, we examined autophagy signals in mice treated with saline or the TNF-α inhibitor and subjected to LCA ligation. Because of the similarity of data on LC3 staining and beclin-1 measurements, we chose to measure LC3 staining as evidence of autophagy. As mentioned earlier, autophagy was seen mostly in the area bordering the infarct in the saline-treated mice (Figure 6B, 6E, and 6F). Unlike apoptosis, LC3 fluorescence density was markedly reduced in the infarct as well as in the border zone in mice treated with the TNF-α inhibitor.

The Western-blot data further confirmed the TUNEL staining and LC3 staining results and showed a significant decrease of LC3-II and an increase of cleaved caspase-3 in the border zones following treatments with TNF-α (Figure 6G through 6I).

Discussion

The presence of extensive inflammation in the hearts of mice subjected to coronary artery ligation has been shown earlier and was confirmed in the present study. Inflammation, documented by accumulation of inflammatory cells and

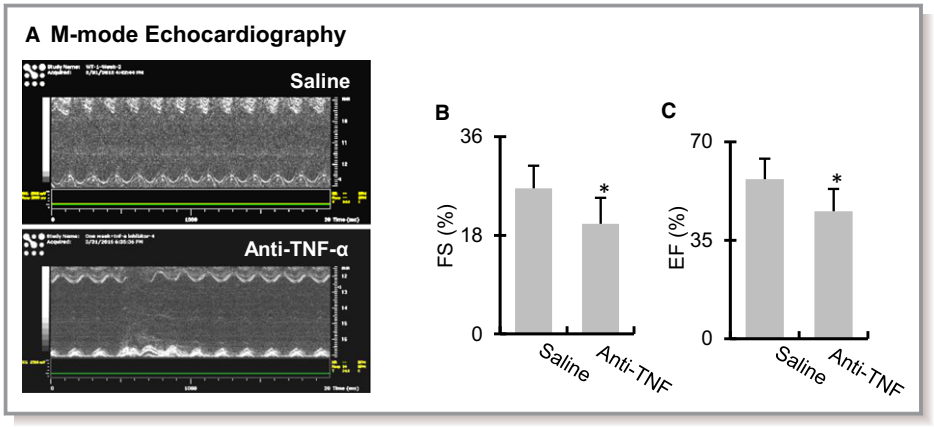

Figure 5. Myocardial function at 1 week following LCA ligation. Mice treated with saline or TNF-α inhibitor. A, Representative M-mode echocardiographic images. B, Quantification of fractional shortening. C, Quantification of ejection fraction. *$P<0.05$ vs the mice given saline solution. EF indicates ejection fraction; FS, fractional shortening; LCA, left coronary artery.

measurement of TNF-α, MCP-1, IL-6, and IL-1β mRNA levels and IL-6 and TNF-α protein levels appeared to be maximal at 1 week after LCA ligation but began to subside thereafter. However, at 4 weeks after LCA ligation, the levels of inflammatory cytokines were still substantially higher in the heart, suggesting a heightened state of inflammation in the chronically ischemic heart. This state of chronic ischemia was characterized by change in heart weight and progressive decline in cardiac contractile function (Figure 1).

There is much interest in the accumulation of different subsets of inflammatory cells with varying functional capability in the ischemic heart.[5,6] In this study we focused on the correlation of inflammation with the phenomena of autophagy and apoptosis. Occurrence of autophagy was maximal early after LCA ligation and but then began to decline soon afterward. We studied inflammation at 1, 2, 3, and 4 weeks after LCA ligation and found autophagy signals to be maximally expressed at 1 week. Although we did not examine this, it is possible that autophagy signals appear at higher levels in the immediate postischemic phase. We studied the phenomenon of autophagy using 2 well-described markers, LC3 and beclin-1, and the changes in both markers were parallel. The conversion of LC3-I into LC3-II is an essential step in autophagosome formation, and the abundance of LC3-II correlates with the number of autophagosomes, and beclin-1 is a well-known key regulator of autophagy.[10,11] It is important to mention that several other investigators have also documented the phenomenon of autophagy in the postischemic period.[12-14]

Autophagy is an important intracellular process in regulating cardiac homeostasis in response to stress. Autophagy is a critical part of normal cell differentiation and morphogenesis.[15] Autophagy has been demonstrated to have a protective role in cardiac response to ischemia by eliminating damaged mitochondria. Several investigators have demonstrated a dramatic increase in autophagy during the reperfusion phase of cardiac ischemia.[16-18] Kanamori et al demonstrated an increase in autophagy activity in the subacute and chronic stages of cardiac ischemia in a mouse model of MI.[19,20] As in our observations, autophagy activity was prominent in the border zone of the infarct compared to remote areas of the myocardium in their study. Further, inhibition of autophagy led to adverse cardiac remodeling following myocardial infarction, suggesting a dynamic role of autophagy in the determination of infarct size and cardiac contractile function. Albeit beneficial, unregulated and sustained autophagy can be deleterious and result in autophagic cell death.[15]

Inflammation is a key regulator for autophagy. An increase in autophagy in response to inflammatory signals such as TNF-α is also well-known.[21,22] Yuan et al reported that LPS and TNF-α both induce autophagy of cardiomyocytes in vitro and in vivo, and enhanced autophagy by rapamycin protect against LPS-mediated myocyte apoptsosis.[23] Wang et al showed a dramatic increase in autophagy signals (LC3 and beclin-1) soon after exposure of cardiomyocytes to hypoxia or Ang II.[7] In the present study the early increase in autophagy signals and a decline afterward paralleled the appearance of proinflammatory signals, suggesting a close link between the 2 phenomena.

Apoptosis, a process of programmed cell death, has also been well described in relation to myocardial ischemia.[24] This phenomenon has been proposed to occur in response to oxidative stress and proinflammatory cytokines. Strategies that limit oxidative stress generally limit infarct size and preserve cardiac function.[25] In the present study we measured 2 well-defined markers of apoptosis—cleaved caspase-3 and TUNEL staining—and the results of the 2 methods were concordant. The novelty of the present study is the description of late onset of apoptosis and its sustenance during the chronic phase of ischemia (versus autophagy, which peaked early and was short-lived). Although the early increase in

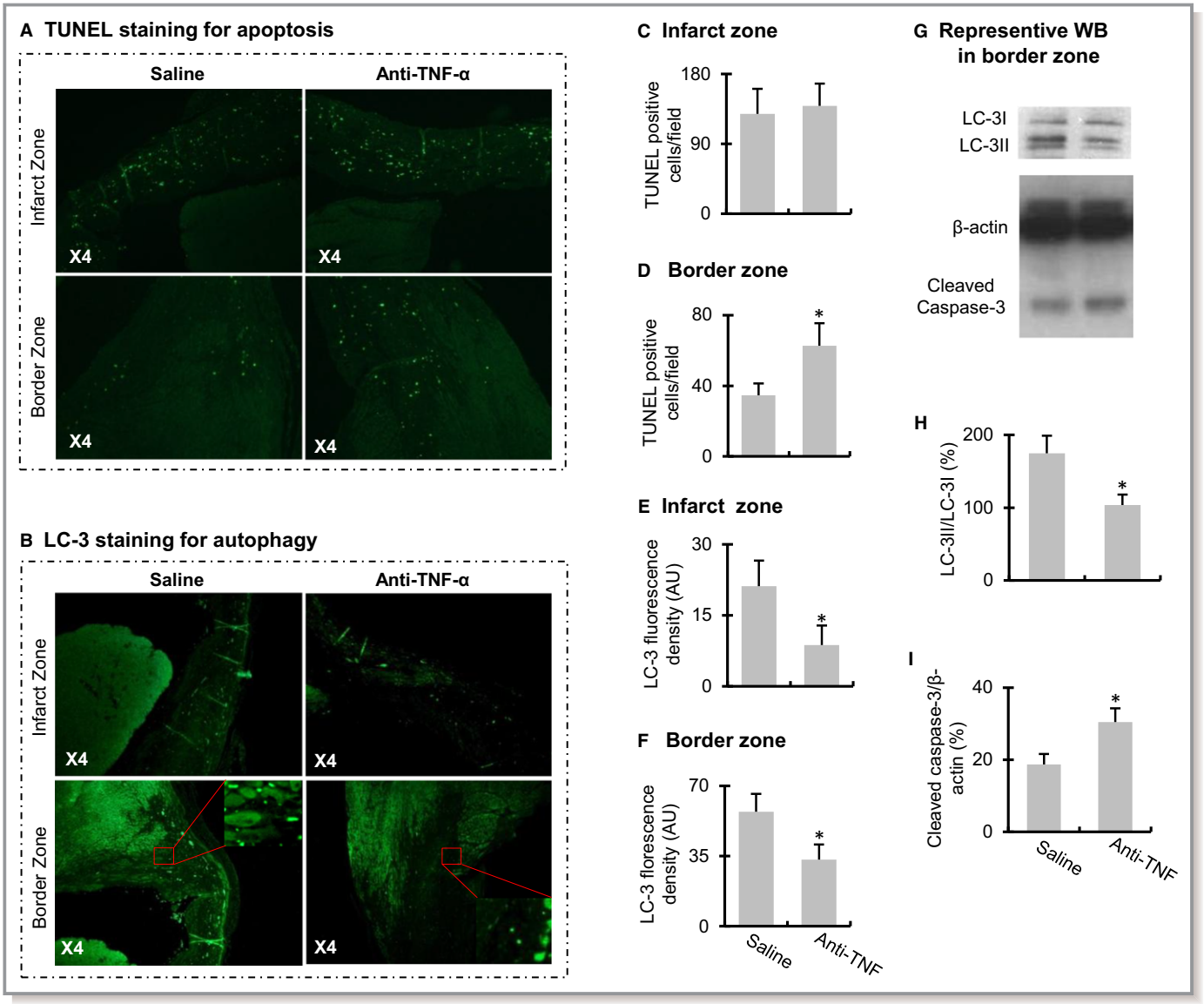

Figure 6. Apoptosis and autophagy in the hearts at 1-week following LCA ligation. Mice treated with saline or TNF-α inhibitor (amplification ×4). A, Apoptosis in the ischemic heart. B, Autophagy in the ischemic heart indicated by LC-3 fluorescence staining. C through F, Quantification of TUNEL-positive cells and LC-3–positive cells in the infarct zones and border zones of LV. G, Representative Western blots showing LC-3I, LC-3II, and cleaved caspase-3 in the ischemic heart. H and I, Quantification of LC-3II/LC-3I and cleaved caspase-3 expression in the border zone. *P<0.05 vs the mice given saline. LCA indicates left coronary artery; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labelling; WB, Western blot.

apoptosis correlated with the appearance of inflammation, there was dissociation between the presence of apoptosis and the sustained increase in inflammation. This observation suggests that factors other than inflammation, such as sustained hypoxia and high levels of Ang II, besides TNF-α, may be responsible for induction of apoptosis. It is of note that in the in vitro studies in cultured cardiomyocytes or vascular smooth muscle cells exposed to hypoxia, Ang II, or oxidized low-density lipoprotein, apoptosis occurred relatively late and was sustained (in contrast to autophagy).

We studied the role of inflammation in the induction of autophagy and apoptosis and determination of cardiac function by treating a group of mice with a selective inhibitor of TNF-α (CAS1049741-03-8). We chose the 1-week post–LCA ligation period when there was maximal inflammation and autophagy and significant apoptosis in the ischemic heart and a marked deterioration of left ventricular contractile function. As expected, there was a dramatic reduction in inflammation in the hearts of mice treated with the TNF-α inhibitor as evident from almost total inhibition of leukocyte accumulation and a drastic reduction in IL-6 expression (immunostaining) and CD68 expression (immunofluorescence) in the infarct area. Importantly, we observed a significant reduction in the occurrence of autophagy signals while the apoptotic cells increased by almost 50%. Reduction in autophagy and an increase in apoptosis resulted in a significant reduction in

worsening of the left ventricular contractile function. Of note, we could not discern a significant change in infarct size with TNF-α inhibition. Thus, it appears that inflammation in the early stages of infarction may not be entirely detrimental. It may be stated that inflammation, by promoting autophagy, may even exert some salutary effect.

Animal studies studying the role of inflammation in the post-MI setting have yielded mixed data. Much of the variability in results may be explained by variability in methodologies. For example, Chen et al in 1994 showed that administration of PB1.3, a monoclonal antibody to P-selectin, reduced oxidant stress, decreased leukocyte accumulation, and preserved cardiac function in dogs subjected to ischemia-reperfusion.[26] However, several other studies have failed to show a beneficial effect of inhibition of inflammation in improving myocardial infarct size or cardiac function.[4,27]

Clinical trials investigating the use of anti-inflammatory drugs in chronic heart failure have been largely unsuccessful in mitigating the process of cardiac remodeling in chronic ischemia. Inhibition of TNF-α has also been studied in patients. In the RENEWAL (Randomized Etanercept Worldwide Evaluation) trial, 74 patients with heart failure and low ejection fraction were treated with etanercept, a recombinant, human TNF receptor that binds to soluble circulating TNF-α and functionally inactivates TNF-α. The trial was stopped prematurely because etanercept had no effect on the occurrence of death or hospitalization. In the ATTACH (Anti-TNF Therapy Against Congestive Heart Failure) trial, 75 patients with HF and reduced ejection fraction were randomized to receive variable doses of infliximab. In this trial, infliximab did not improve left ventricular ejection fraction. Importantly, high doses of infliximab were associated with increased risk of death and hospitalization from HF. Further clinical studies are also required to address whether TNF-α inhibitor (CAS1049741-03-8) affects cardiac function of patients with heart failure.

Our study provides a clue as to how anti-inflammatory drug therapy, in particular anti–TNF-α therapy, may adversely affect cardiac function. Inhibition of autophagy with unabated occurrence of apoptosis of myocytes may explain at least in part the worsening of left ventricular function after coronary artery occlusion.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Figure S1. Relationship between inflammation, autophagy and apoptosis at 1 week following LCA ligation. (a). Relationship between beclin-1 and TNF-α. **(b).** Relationship between cleaved-caspase-3 and TNF-α. **(c).** Relationship between caspase-3 and beclin-1.