Pharmacologic Inhibition of Myeloid Differentiation Factor 88 (MyD88) Prevents Left Ventricular Dilation and Hypertrophy After Experimental Acute Myocardial Infarction in the Mouse

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Background: Myeloid differentiation factor 88 (MyD88) is an endogenous adaptor protein that coordinates the inflammatory response to agonists of the Toll-like receptor and interleukin-1 receptor families. This particular response is activated following myocardial ischemia and infarction and may represent a viable target for pharmacologic inhibition. The current study tested MyD88 inhibitors in a murine model of nonreperfused acute myocardial infarction (AMI).

Methods: AMI was induced by permanent ligation of the left coronary artery. Adult, male, Imprinting Control Region mice were randomized to daily injections with 1 of 2 MyD88 pharmacologic inhibitors (ST2825 25 mg/kg or IMG2005 1 mg/kg), saline, or pretreatment with MyD88-targeted silencing small interfering RNA (siRNA) or scrambled nontargeted siRNA (n = 6 for each group). Echocardiography was performed at baseline and 7 days after surgery to evaluate pathologic cardiac enlargement.

Results: Pharmacologic inhibition of MyD88 with ST2825 or IMG2005) and MyD88-targeted siRNA protected against left ventricular (LV) dilatation (reduced LV end-systolic and LV end-diastolic diameter) and hypertrophy. This protection occurred despite no measurable reduction in infarct size.

Conclusions: Pharmacologic MyD88 inhibition protects against pathologic LV remodeling without altering infarct scar formation. MyD88 may be a viable target for pharmacologic inhibition in AMI.

Key Words: MyD88, acute myocardial infarction, heart failure, inflammation

(*J Cardiovasc Pharmacol*™ 2010;55:385–390)

Received for publication October 28, 2009; accepted December 29, 2009.

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Supported by an A. D. Williams Research Grant to Dr Van Tassell and an American Heart Association Beginning Grant-in-Aid to Dr Abbate.

Disclosures: None.

The authors reports no conflicts of interest.

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INTRODUCTION

Acute myocardial infarction (AMI) is a primary cause of morbidity and mortality in the United States and worldwide.[1] Current treatments aim to prompt reperfusion of ischemic tissue and reduction of myocardial oxygen requirements. Despite treatment advances, complete reperfusion is not always achievable and restoration of arterial patency does not ensure reperfusion of myocardial tissue, and patient with poor reperfusion are at higher risk for pathologic cardiac enlargement, heart failure, and death.[2,3] During the acute phase of infarction, damage to myocardial tissue promotes the release of proinflammatory mediators within the myocardium.[4] This inflammatory response is associated with increased myocardial cell death (apoptosis) and pathologic remodeling of the heart during the postinfarction period.[4,5]

The complexity and redundancy of the inflammatory response may represent a limitation to the success of targeted anticytokine therapy in AMI and heart failure.[6] Independent lines of research have described a role of interleukin (IL)-1,[7,8] IL-18,[9,10] IL-33,[11,12] and Toll-like receptor agonists[13–15] in AMI and heart failure. These cytokines/mediators all share the same family of receptor/accessory proteins that share a sequence homology known as the Toll/IL-1 receptor (TIR) domain.[16,17] The myeloid differentiation factor 88 (MyD88) is an innate adaptor protein that binds to the TIR domain to coordinate the cellular response to the different agonists.[16,17] Upon binding with one of the TIR receptor complexes, MyD88 homodimers activate the IL-1 associated kinase (IRAK) complex which phosphorylates downstream kinases and transcription factors [ie, nuclear factor κB (NF-κB)].

Several reports have linked MyD88 and the innate immune response to myocardial response following ischemia. Mice with genetic deletion of MyD88[18,19] or IRAK-4[20] expression have smaller infarcts in AMI models induced by transient ischemia followed by reperfusion or permanent coronary ligation, respectively. From a translational standpoint, it is important to consider that effective reperfusion is not obtained in all patients presenting with AMI; many patients present late for reperfusion or have evidence of poor myocardial reperfusion. These are the patients who incur the highest risk of developing pathologic cardiac enlargement and heart failure and for whom there is an urgent need for adjunctive therapy.[2]

The potential of MyD88 inhibition to prevent heart failure following AMI has not been investigated. We hypothesized that pharmacologic inhibition of MyD88 would provide advantageous TIR blockade and prevent pathologic cardiac enlargement in a model of AMI without reperfusion, representing the basis for a novel treatment target for the prevention of heart failure following AMI. We hereby report the effects of 2 novel pharmacologic MyD88 inhibitors[22,23] that ameliorated cardiac enlargement following AMI.

MATERIALS AND METHODS

Animals

Adult male out-bred Imprinting Control Region (ICR) mice (8 weeks old, 25–30 g) were supplied by Harlan Sprague–Dawley (Indianapolis, IN). All animal experiments were conducted under the guidelines on humane use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996). The study protocol was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Drugs

ST2825 is a small molecule that mimics the TIR domain of the MyD88 protein and inhibits homodimerization, provided at no cost by Sigma Tau (Pomezia, Italy). IMG2005 is a synthetic oligopeptide with a structure similar to ST2825 that also inhibits homodimerization, purchased from Imgenex Corporation (San Diego, CA). As IMG2005 had not been previously evaluated for in vivo use, the initial dose (1 mg/kg) was extrapolated from oligopeptides of similar size that share a common antennapedia sequence with IMG2005.[21,22] All drugs were given intraperitoneally.

Small Interfering RNA

A previously validated protocol was used to explore decreasing doses of small interfering RNA (siRNA; 1.5, 1.0, 0.45, 0.15, 0.1 mg/g).[24] The siRNA was purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA), reconstituted in RNAase-free water, mixed with an equal volume of siPORT amine (Applied Biosystems, Austin, TX, room temperature, 30 minutes) to promote cell internalization, and then injected into the mice. A scrambled nontargeted siRNA sequence was used as a control. Western blots using primary antibodies raised against MyD88 (produced in rabbit, 1:500 dilution, Sigma-Aldrich Corp., St. Louis, MO) and β-actin (produced in mouse clone AC-40, purified immunoglobulin, dilution 1:5000, Sigma Aldrich Corp.) were performed to determine silencing efficacy. Proteins were extracted from the heart tissue homogenate (Ripa buffer and proteases cocktail inhibitor, Sigma Aldrich Corp.), transferred to polyvinylidene fluoride membranes (0.45 μm pore size), and incubated with 3% milk for 2 hours at room temperature. Chemiluminescence detection was performed with Immobilon Western Chemiluminescent horse radish peroxidase substrate (ECL, Millipore, Billerica, MA) and quantified using Scion Image software (Scion Corp., Frederick, MD).

Interleukin-6 Plasma Levels

Interleukin-6 levels were used as a surrogate of NF-κB activity following IL-1β (3 μg/kg) challenge.[25] Blood was drawn by direct cardiac puncture at sacrifice 4 hours after IL-1β challenge, transferred to heparin tubes and centrifuged at 2000*g* for 15 minutes. The supernatant was collected and stored at −20°C until subsequent analysis. A quantitative sandwich enzyme immunoassay (Quantikine, RnD Systems, Minneapolis, MN) was used according to the manufacturer's instructions.

Myocardial Infarction Protocol

The animals were anesthetized with injection of pentobarbital (70 mg/kg IP), intubated orotracheally, and ventilated on a positive-pressure ventilator. The tidal volume was set at 0.2 mL, and the respiratory rate was adjusted to 133 cycles/min. All surgical procedures were carried out under sterile conditions, as described previously.[7] A left thoracotomy was performed at the fourth intercostal space, and the heart was exposed by stripping the pericardium. The left descending coronary artery was then identified and ligated with a 7.0 silk ligature.

Experimental Groups

Mice underwent surgical coronary artery ligation to simulate and random assignment to 1 of 5 treatment groups (Fig. 1, n = 6 per group): normal saline (control), pharmacologic MyD88 inhibitor (ST2825), pharmacologic inhibitor (IMG2005), MyD88-targeted siRNA, and scrambled nontargeted siRNA. An additional 5 groups of mice (n = 4 per group) underwent sham operation and treatment. All mice

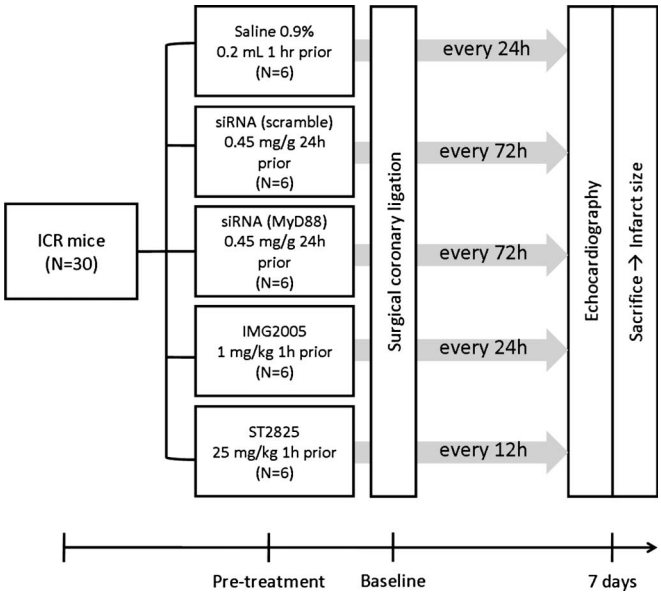

FIGURE 1. Experimental groups. ICR mice were divided into 1 of 5 treatment groups (N = 6 for each group): saline 0.9%, siRNA (scramble), siRNA (MyD88), IMG2005 (MyD88 inhibitor), and ST2825 (MyD88 inhibitor). Mice received a single treatment prior to surgical coronary artery ligation and continued treatment for 7 days after surgery. A separate 5 groups of mice underwent the same treatments and procedure but without coronary artery ligation (sham).

underwent echocardiography at baseline and 7 days after infarction. Upon completion of echocardiography, mice were sacrificed (70 mg/kg pentobarbital injection) and the hearts immediately removed and fixed in 10% formalin (at least 48 hours) for subsequent histologic evaluation.

Doppler Echocardiography

Doppler echocardiography was performed using the Vevo770 imaging system (VisualSonics Inc, Toronto, Canada) to obtain measurements at baseline and 7 days later, immediately before sacrificing the animal. Light anesthesia was used during the measurement with injection of pentobarbital (30 mg/kg IP). A liquid depilatory was used to remove the fur and the mice were placed on a heated platform. Ultrasound gel was used on the thorax to optimize visibility during the exam. A 30-MHz probe was utilized to obtain 2-dimensional and M-mode images from the parasternal short-axis view at the level of the papillary muscles, according to the recommendations of the American Society of Echocardiography.[26] M-mode images of the LV were obtained to measure systolic and diastolic wall thickness (anterior and posterior) and LV end-systolic and end-diastolic diameters (LVESD and LVEDD, respectively). LV fractional shortening was calculated as (LVEDD − LVESD)/LVEDD × 100. LV ejection fraction was calculated with the Teichholz formula. LV mass was calculated based on LVEDD, posterior wall diastolic thickness (PWDT), and anterior wall diastolic thickness (AWDT) using the Deveraux formula as $0.8 \times [1.04(\text{LVEDD} + \text{PWDT} + \text{AWDT})^3 - (\text{LVEDD})^3] + 0.6$ g.[26] The investigators performing and reading the echocardiogram were blinded to treatment allocation.

Infarct Scar

Myocardial fibrosis was measured 7 days after infarction to assess infarct scar dimensions in the LV. Heart sections were stained with Masson trichrome (Sigma-Aldrich). The areas of fibrosis and the whole left ventricle were determined by computer morphometry using a Bioquant imaging software. The ratio was used to compute scar area expressed as a percentage of the left ventricle.

Statistics

All measurements are expressed as group means ± SE. Discrete variables were presented as absolute and percent value. Differences between the groups were analyzed using the 1-way ANOVA and changes in echocardiography and infarct size were analyzed using the random effects ANOVA for repeated-measures to determine the main effect of time, group, and time-by-group interaction, using Dunnett test for comparison between the different groups. Statistical differences were considered significant if the P value was < 0.05. All authors had full access to the data and take responsibility for its integrity.

RESULTS

Protein Expression

Western blot analysis showed effective suppression of MyD88 expression in mice treated with MyD88-targeted siRNA (at 24 hours after siRNA administration) compared with those treated with nontargeted scrambled siRNA (Fig. 2). MyD88 expression remained suppressed for up to 72 hours and returned to baseline at approximately 96 hours after single-dose siRNA administration (data not shown).

Interleukin-6 Levels as Surrogate for NF-κB Activity

IL-1β (3 μg/kg) led to a significant increase in plasma IL-6 levels at 4 hours (Fig. 3). Mice pretreated with MyD88-targeted siRNA (24 hours earlier) had significantly lower IL-6 levels after IL-1β challenge than controls receiving IL-1β alone and mice pretreated with scrambled nontargeted siRNA followed by IL-1β ($P < 0.05$ for both comparison). Similarly, mice pretreated with ST2825 (25 mg/kg) or IMG2005 (1 mg/kg) had significantly lower IL-6 levels following IL-1β challenge compared with mice receiving IL-1β without pretreatment (Fig. 3).

Cardiac Enlargement Following AMI

Changes in cardiac dimensions after AMI are surrogate endpoints for heart failure-related morbidity and mortality.[27,28] Figure 4 shows baseline and 7-day LVEDD and LVESD in the 5 groups of mice with AMI. As expected, AMI led to significant enlargement of LV diameter in all treatment groups. However, MyD88-targeted siRNA led to a 44% attenuation of LVEDD dilation [+1.28 mm (scramble) versus +0.72 mm (siRNA), $P < 0.05$] and a 29% attenuation in LVESD dilation [+2.10 mm (scramble) versus 1.50 mm (siRNA), $P < 0.05$] when compared with mice receiving scrambled nontargeted siRNA solutions.

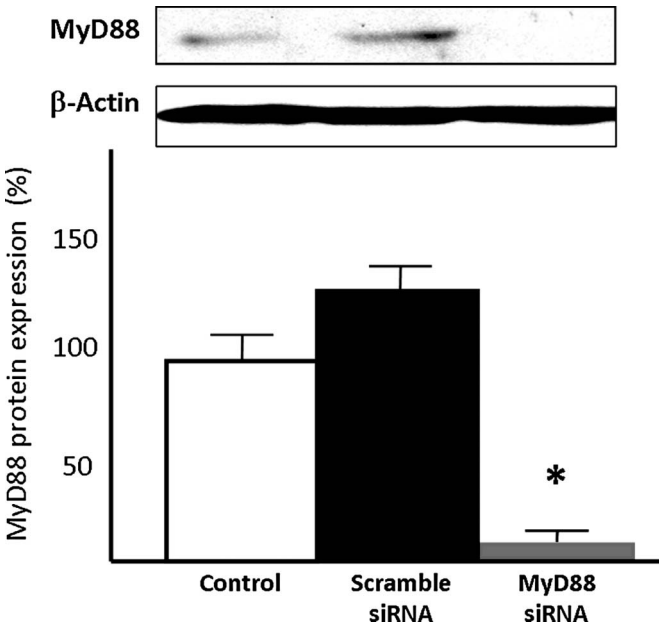

FIGURE 2. MyD88 protein interference. Mice were divided into 3 groups (N = 4 per group): no treatment (control); injection with nontargeted siRNA (scramble) 0.45 mg/g IP Q72 hours; and targeted siRNA (MyD88) 0.45 mg/g IP Q72 hours. Mice were sacrificed after 7 days and the hearts analyzed for MyD88 protein expression by Western blot.

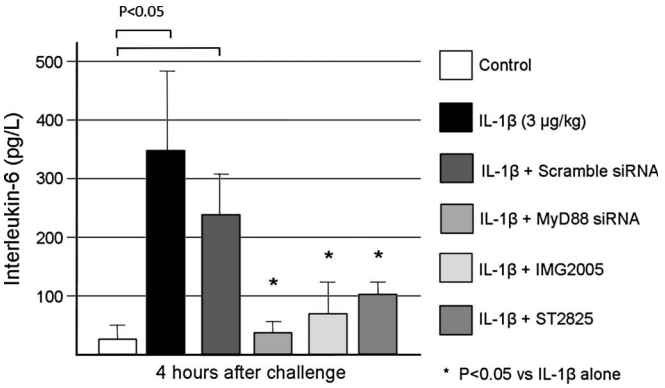

FIGURE 3. IL-6 concentration as a surrogate for NF-κB activity. Mice received injection with IL-1β (3 μg/kg IP) to stimulate IL-6 production followed by blood sampling at 4 hours (N ≥ 4 per group). IL-6 concentration was determined by quantitative ELISA. Pretreatment with MyD88-targeted siRNA or pharmacologic MyD88 inhibitors blunted IL-1 effects on IL-6 concentration.

Pharmacologic inhibition of MyD88 with ST2825 or IMG2005 also led to similar attenuations of changes in LVEDD [+1.28 mm (saline), +0.70 mm (ST2825, −44%), +0.88 mm (IMG2005, −31%)] and LVESD [+2.03 mm (saline), +1.56 mm (ST2825, −23%), +1.71 mm (IMG2005, −15%)] when compared with mice receiving control saline treatment. The changes in LVEDD and LVESD were not statistically different comparing mice treated with ST2825, IMG2005, or MyD88-targeted siRNA ($P > 0.05$ for all comparisons). LV ejection fraction, on the other hand, was significantly reduced by approximately 60% in all groups without differences comparing the control group with any other intervention groups (data not shown). No interval changes in LV dimensions or function were noted in any of the sham-operated mice groups (data not shown).

In terms of hypertrophy, saline-treated mice experienced a significant change in LV mass as assessed by echocardiography at 7 days after coronary ligation surgery [90 ± 3 mg (baseline) versus 110 ± 4 mg (7 days), $P < 0.001$]. MyD88 blockade with MyD88-targeted siRNA, IMG2005 or ST2825 significantly attenuated hypertrophy [95 ± 14 mg (siRNA), 82 ± 12 mg (ST2825), 96 ± 5 mg (IMG2005), $P < 0.05$ versus for all groups versus normal saline]. Mice treated with scrambled nontargeted siRNA showed LV hypertrophy not different from normal saline-treated mice (109 ± 8 mg).

Infarct Size

Treatment with MyD88-targeted siRNA, ST2825, or IMG2005 did not affect LV infarct scar formation (as measured by trichrome fibrosis staining at 7 days) when compared with saline control-treated mice or mice treated with scrambled nontargeted siRNA (all P values > 0.05, Fig. 5).

DISCUSSION

Myocardial ischemia and ensuing cell death during AMI initiates a complex inflammatory response characterized by multiple mediators and pathologic cardiac enlargement.[4,5] The results of the current study identify MyD88, an adaptor protein tightly linked to innate immunity, as a potential target for treatment. Indeed, MyD88 inhibition using pharmacologic inhibitors and silencing RNA led to significantly less cardiac enlargement in a model of AMI due to permanent left coronary ligation in the mouse. From a pathophysiologic standpoint, the similarity of the effects obtained by blocking MyD88 directly and by blocking the individual TIR agonists IL-1, IL-18, and

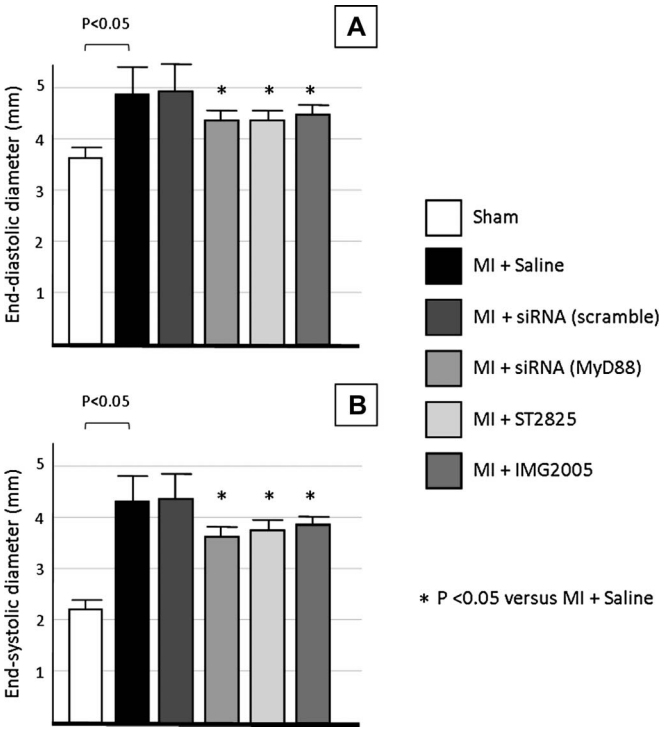

FIGURE 4. Echocardiographic measurement 7 days after coronary artery ligation. Pharmacologic MyD88 inhibition attenuated the change in echocardiographic measures of LVEDD (A) and LVESD following permanent coronary artery ligation surgery.

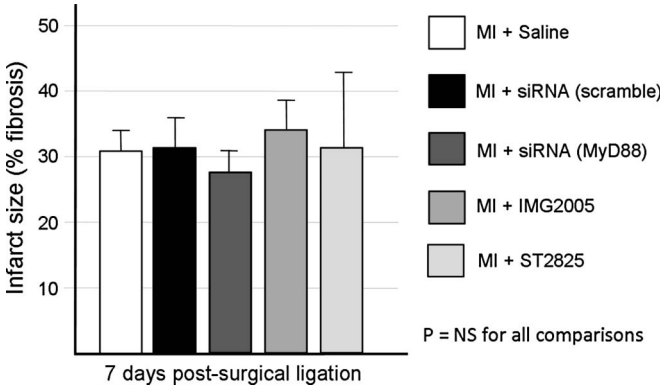

FIGURE 5. Infarct size. MyD88-targeted pharmacologic inhibition did not affect infarct scar formation (collagen deposition) at 7 days after coronary artery ligation.

toll-like receptor suggests that MyD88 modulates cardiac remodeling at a signal convergence point downstream of such agonists.[16,17] From a clinical standpoint, there is an urgent need to identify viable therapeutic targets to modulate inflammation after AMI.[2–4,29] As ventricular dilation is an established predictor of progression to heart failure, the prevention of cardiac enlargement by pharmacologic inhibition of MyD88 may represent a completely novel approach to prevent heart failure.[32] Such a hypothesis would clearly require validation in human clinical trials.

The finding of a significant role for MyD88 signaling in our model of nonreperfused AMI is in keeping with prior observation of models of AMI of lesser severity (ie, transient ischemia).[18,19] Nevertheless, some differences in the findings are obvious and need to be addressed. First, MyD88 inhibition by either pharmacologic inhibition or silencing RNA did not show an infarct sparing effect, whereas previous findings describe a smaller infarct in MyD88-deficient mice.[18,19] There are several differences in the experimental setting that may explain this discrepancy: first, the earlier studies used genetically engineered mice that may develop compensatory alterations in parallel signaling pathways to counteract permanent abrogation of MyD88 signaling. In this case, transient interruption of MyD88 (using pharmacologic inhibitors and siRNA) may constitute a more translatable model of MyD88 blockade than knockout models with permanent MyD88 deletion. Second, different models of AMI have been used (transient ischemia versus prolonged ischemia) that vary in their pathophysiologic evolution and inflammatory response.[30] The effects of transient ischemia/reperfusion in healthy mice may be substantially different from those of plaque rupture, thrombosis, and reperfusion in chronically atherosclerotic human patients. Third, pharmacologic inhibitors and silencing RNA are not perfectly specific and may have influenced pathways beyond MyD88 that our assays were not designed to detect. The extent and magnitude of these unanticipated effects are, by definition, unknown and difficult to quantify.

The effects of MyD88 inhibition in our model of nonreperfused AMI model due to coronary ligation is consistent with the findings of more favorable cardiac remodeling, independent of infarct size in mice with genetic deletion of the IRAK-4 gene subjected to permanent ligation.[21]

In the current study, MyD88 interruption did not elicit any change in infarct size. This finding may be partially attributed to the model of permanent coronary ligation, which may limit the potential for infarct sparing. Nonetheless, the finding of reduced LV enlargement independent of infarct-sparing effects may prove to be of significant translational value. Due to the proficiency of contemporary reperfusion techniques, infarct size is primarily determined by the success of coronary reperfusion. Moreover, patients with poor reperfusion are at highest risk of heart failure and may obtain the most benefit from MyD88 inhibition.[30]

The exact mechanism by which MyD88 inhibition prevents cardiac enlargement following AMI is also not established. Our observations confirm MyD88 as an important signaling node but do not clarify the downstream effects. Further studies are needed to explore the effects of MyD88 at the cellular and molecular levels. For example, MyD88-independent signaling of TIR receptors has been described in pathologies other than cardiac.[16,17] Whether these MyD88-independent pathways are involved or not in cardiac remodeling is unclear.

The current finding that MyD88 inhibition may prevent cardiac enlargement and hypertrophy independent of any benefit on LV function highlights the potential dissociation between cardiac function and survival. This may correlate with previous human clinical trials, such as the Vasodilator Heart Failure Trial study, in which enalapril led to a lesser improvement in ejection fraction but to a greater survival than hydralazine/isosorbide.[27,31] The divergence of effect on cardiac remodeling and on function in our study may however be due, in part, to the short duration of follow-up (1 week) after infarction. Indeed, the initial increase of LV volumes after AMI serves the purpose of preserving the stroke volume; whereas the late LV dilation becomes a mechanism of reduced ejection fraction and progression to heart failure.[27,31,32] Our findings also suggest that some degree of hypertrophy is essential to maintain LV function after AMI and the lack of compensatory hypertrophy following MyD88 blockade may have contributed to the resultant neutral effect on LV function. Although clinical measurements of LV function do predict outcomes after AMI, clinical studies of angiotensin-converting enzyme inhibitors in post-AMI patients found that LV dilation was a more powerful predictor of mortality than LV function.[31,32]

In summary, we found that pharmacologic inhibition of MyD88 in vivo attenuates pathologic LV dilation and hypertrophy in a mouse model of nonreperfused AMI independent of infarct size. MyD88 is an important adaptor protein at the convergence of multiple proinflammatory pathways in innate immunity. Our findings suggest that MyD88 inhibition may become a novel pharmacologic target for future translational investigation for the prevention of heart failure following AMI.

REFERENCES

1. National Heart Lung and Blood Institute. *Morbidity and Mortality: 2007 Chart Book on Cardiovascular and Lung Diseases*. Bethesda, MD: NIH; 2007.
2. Niccoli G, Burzotta F, Galiuto L, et al. Myocardial no-reflow in humans. *J Am Coll Cardiol*. 2009;54:281–292.
3. Abbate A, Kontos MC, Biondi-Zoccai GG. No-reflow: the next challenge in treatment of ST-elevation acute myocardial infarction. *Eur Heart J*. 2008;29:1795–1797.
4. Frangogiannis N. The immune system and cardiac repair. *Pharm Res*. 2008;58:88–111.
5. Abbate A, Bussani R, Amin MS, et al. Acute myocardial infarction and heart failure: role of apoptosis. *Int J Biochem Cell Biol*. 2006;38:1834–1840.
6. Mann DL. Targeted anticytokine therapy and the failing heart. *Am J Cardiol*. 2005;95(Suppl):9C–16C.
7. Abbate A, Salloum FN, Vecile E, et al. Anakinra, a recombinant human interleukin-1 receptor antagonist, inhibits apoptosis in experimental myocardial infarction. *Circulation*. 2008;117:2670–2683.
8. Bujak M, Dobaczewski M, Chatila K, et al. Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling. *Am J Pathol*. 2008;173:57–67.
9. Venkatachalam K, Prabhu SD, Reddy VS, et al. Neutralization of Interleukin-18 ameliorates ischemia/reperfusion induced myocardial injury. *J Biol Chem*. 2009;284:7853–7865.

10. Yu Q, Vasquez R, Khojeini EV, et al. IL-18 induction of osteopontin mediates cardiac fibrosis and diastolic dysfunction in mice. *Am J Physiol Heart Circ Physiol*. 2009;297:H76–H85.
11. Sanada S, Hakuno D, Higgins LJ, et al. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest*. 2007;117:1538–1549.
12. Bartunek J, Delrue L, Van Durme F, et al. Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J Am Coll Cardiol*. 2008;52:2166–2174.
13. Riad A, Jagar S, Sobirey M, et al. Toll-like receptor-4 modulates survival by induction of left ventricular remodeling after myocardial infarction in mice. *J Immunol*. 2008;180:6954–6961.
14. Shishido T, Nozaki N, Yamaguchi S, et al. Toll-like receptor-2 modulates ventricular remodeling after myocardial infarction. *Circulation*. 2003; 108:2905–2910.
15. Kim SC, Ghanem A, Stapel H, et al. Toll-like receptor 4 deficiency: smaller infarcts, but no gain in function. *BMC Physiol*. 2007;7:5–14.
16. O'Neil LAJ, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signaling. *Nat Rev Immunol*. 2007;7:353–364.
17. Daun JM, Fenton MJ. Interleukin-1/Toll receptor family members: receptor structure and signal transduction pathways. *J Interf Cytokine Res*. 2000;20:843–857.
18. Feng Y, Zhao H, Xu X, et al. Innate immune adaptor MyD88 mediates neutrophil recruitment and myocardial injury after ischemia-reperfusion in mice. *Am J Physiol Heart Circ Physiol*. 2008;295:H1311–H1318.
19. Hua F, Ha T, Ma J, Gao X, et al. Blocking the MyD88-dependent pathway protects the myocardium from ischemia/reperfusion injury in rat hearts. *Biochem Biophys Res Commun*. 2005;338:1118–1125.
20. Maekawa Y, Mizui N, Chan A, et al. Survival and cardiac remodeling after myocardial infarction are critically dependent on the host innate immune Interleukin-1 receptor-associated kinase-4 signaling. A regulator of bone marrow-derived dendritic cells. *Circulation*. 2009. [Epub ahead of print].
21. Loiarro M, Capolunghi F, Fantò N, et al. Pivotal Advance: Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound. *J Leukoc Biol*. 2007;82:801–810.
22. Loiarro M, Sette C, Gallo G, et al. Peptide-mediated interference of TIR domain dimerization in MyD88 inhibits interleukin-1 dependent activation of NF-κB. *J Biol Chem*. 2005;280:15809–15814.
23. Derossi D, Joliot AH, Chassaings G. The third helix of the antennapedia homeodomain translocates through biological membranes. *J Biol Chem*. 1994;269:10444–10450.
24. Natarajan R, Salloum FN, Fisher BJ, et al. Hypoxia inducible factor-1 activation by prolyl4-hydroxylase-2 gene silencing attenuates myocardial ischemia reperfusion injury. *Circ Res*. 2006;98:133–140.
25. Parikh AA, Salzman AL, Kane CD, et al. IL-6 production in human intestinal epithelial cells following stimulation with IL-1 beta is associated with activation of the transcription factor NF-kappa B. *J Surg Res*. 1997; 69:139–144.
26. Gardin J, Adams D, Douglas P, et al. Recommendations for a standardized report for adult transthoracic echocardiography: a report from the American Society of Echocardiography's Nomenclature and Standards Committee and Task Force for a Standardized Echocardiography Report. *J Am Soc Echocardiogr*. 2002;15:275–290.
27. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol*. 2000;35:569–582.
28. White HD, Norris RM, Brown MA, et al. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation*. 1987;29:44–51.
29. Gilroy DW, Lawrence T, Perretti M, et al. Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov*. 2004;3:401–416.
30. Vandervelde S, van Amerongen MJ, Tio RA, et al. Increased inflammatory response and neorevascularization in reperfused vs nonreperfused murine myocardial infarction. *Cardiovasc Pathol*. 2006;16:83–90.
31. Cintron G, Johnson G, Francis G, et al. Prognostic significance of serial changes in left ventricular ejection fraction in patients with heart failure. The V-HeFT VA Cooperative studies group. *Circulation*. 1993;87:V-117–V-123.
32. Anand IS, Florea VG, Fisher L. Surrogate end points in heart failure. *J Am Coll Cardiol*. 2002;39:1414–1421.