Interleukin-1 Trap Attenuates Cardiac Remodeling After Experimental Acute Myocardial Infarction in Mice

Benjamin W. Van Tassell, PharmD,†‡ Amit Varma, MD,* Fadi N. Salloum, PhD,* Anindita Das, PhD,* Ignacio M. Seropian, MD,*†‡ Stefano Toldo, MS,*† Lisa Smithson, BS,† Nicholas N. Hoke, BS,* Vinh Q. Chau, BS,* Roshanak Robati, MD,* and Antonio Abbate, MD, PhD*†*

Background: Interleukin-1 (IL-1) is an inflammatory cytokine that responds as an acute phase reactant during acute myocardial infarction. Conflicting data describe the role of anti-IL-1 interventions to reduce cardiac remodeling after AMI. IL-1 Trap is a modified recombinant fusion protein that binds circulating IL-1. Our study evaluated the effects of murine IL-1 Trap on cardiac remodeling after AMI resulting from permanent surgical coronary artery ligation.

Methods: Mice received treatment with intraperitoneal injection of murine IL-1 Trap (1 mg/kg [n = 5], 5 mg/kg [n = 5], or 30 mg/kg [n = 5]) or NaCl 0.9% (saline; n = 10) every 48 hours after surgery. Transthoracic echocardiography was performed at baseline and 7 days after surgery. Inhibition of IL-1 signaling was determined by measurement of IL-6 plasma levels (enzyme-linked immunosorbent assay) after IL-1β injection. Apoptosis (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) was measured in murine heart samples and in a primary culture of murine cardiomyocytes.

Results: Mice treated with 5 mg/kg or 30 mg/kg IL-1 Trap had more favorable cardiac remodeling and echocardiographic assessment of infarct size at 7 days compared with saline ($P < 0.05$ for each comparison). Treatment with IL-1 Trap also reduced apoptosis and IL-6 levels compared with saline treatment.

Conclusions: IL-1 Trap ameliorates cardiac remodeling and reduces cardiomyocyte apoptosis after experimental acute myocardial infarction in the mouse.

Key Words: interleukin-1, IL-1 Trap, Rilonacept, myocardial infarction, inflammation, cardiac remodeling

(*J Cardiovasc Pharmacol*™ 2010;55:117–122)

Received for publication August 11, 2009; accepted October 20, 2009.

From the *Pauley Heart Center, †Victoria Johnson Center; and ‡School of Pharmacy, Virginia Commonwealth University, Richmond, VA.

This work was supported in part by an American Heart Association Beginning Grant-in-Aid (Mid-Atlantic Affiliate) to Dr. Abbate.

B.W.V.T., A.V., and F.N.S. equally contributed to the realization of this article.

The authors report no conflicts of interest.

Reprints: Antonio Abbate, MD, PhD, Assistant Professor of Medicine, Division of Cardiology/VCU Pauley Heart Center, Virginia Commonwealth University, 1200 East Broad Street, West Hospital, 10th Floor, East Wing, Room 1041 PO Box 980281, Richmond, VA 23298-0281 (e-mail: aabbate@mcvh-vcu.edu).

INTRODUCTION

Acute myocardial infarction (AMI) is a primary cause of morbidity and mortality in the United States and worldwide.[1] During AMI, multiple proinflammatory mediators are released within the myocardium that are associated with apoptosis, pathologic ventricular remodeling, and progression to heart failure.[2–5] Interleukin-1 (IL-1) is the prototypal inflammatory cytokine and signals through a devoted IL-1 Type 1 receptor ($IL\text{-}1R_1$).[6] Both IL-1 and its naturally occurring antagonist, IL-1 receptor antagonist (IL-1Ra), respond as acute phase reactants during AMI and remain elevated in patients with heart failure.[6–8]

Previous studies reveal conflicting evidence for the role of IL-1 in postinfarction remodeling. Two independent reports describe favorable outcomes through suppression of IL-1 signaling in experimental models of AMI. In one study, mice with a genetic deletion of the $IL\text{-}1R_1$ ($IL\text{-}1R_1^{-/-}$) experienced reduced fibrotic response and attenuated ventricular remodeling after myocardial ischemia/reperfusion injury.[9] A second study reported reduced ventricular remodeling, reduced cardiomyocyte apoptosis, and improved survival in both mice and rats receiving recombinant human IL-1 receptor antagonist (rhIL-1Ra) after permanent coronary ligation surgery.[10] However, these findings conflict with a prior report that described worsening ventricular remodeling and cardiac rupture in mice receiving monoclonal hamster anti-mouse IL-1β antibody after permanent coronary ligation.[11]

IL-1 Trap is a novel fusion protein that combines the soluble domains of the $IL\text{-}1R_1$ and IL-1R accessory protein ($IL\text{-}1R_{AcP}$) to bind IL-1α and IL-1β and inhibit agonist activation of the membrane $IL\text{-}1R_1$. A humanized analog of IL-1 Trap Rilonacept (Regeneron Pharmaceuticals Inc., Tarrytown, NY) is Food and Drug Administration-approved for the treatment of inflammatory disorders. The current study aimed to test the effects of murine IL-1 Trap on ventricular remodeling and cardiomyocyte apoptosis after experimental AMI and confirm the viability of IL-1 blockade as a therapeutic target.

MATERIALS AND METHODS

Animals and Drugs

Adult male outbred ICR mice were purchased from Harlan Laboratories (Indianapolis, IN). All animal experiments were conducted under the guidelines on humane use and care of laboratory animals for biomedical research published by the US National Institutes of Health (National Institutes of

Health Publication No. 85-23, revised 1996). The study protocol was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Murine IL-1 Trap was provided at no cost by Regeneron Pharmaceuticals Inc. (Tarrytown, NY) and the initial dosing (5 mg/kg) was based on manufacturer recommendations.

Interleukin-6 Response to Exogenous Interleukin-1β

To test the blocking effect of IL-1 Trap, we determined the in vivo IL-6 response to exogenous IL-1 in healthy mice. A group of male ICR mice were randomly assigned to pretreatment with 5 mg/kg IL-1 Trap (n = 4) or saline (n = 4) followed by intraperitoneal injection of 100 ng recombinant murine IL-1β (3 μg/kg, Millipore, Billerica, MA). A third group of mice (n = 4) did not receive either IL-1 Trap nor IL-1. Four hours later, a whole blood sample was drawn from the heart in heparin tubes (at the time of euthanasia) and centrifuged at 2000 *g* for 15 minutes. The supernatant plasma was collected and stored at –20°C until subsequent analysis. IL-6 response was measured by quantitative sandwich enzyme immunoassay (Quantikine; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Acute Myocardial Infarction Protocol

Mice underwent experimental myocardial infarction as previously described.[10] Briefly, mice were anesthetized with intraperitoneal (IP) pentobarbital (70 mg/kg), intubated orotracheally, and ventilated on a positive-pressure ventilator (tidal volume = 0.25 mL, respiratory rate = 133 cycles/minute). 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. Sham-operated mice underwent the same surgical procedure without coronary ligation. For the infarction protocol, male ICR mice were randomly assigned to treatment with 5 mg/kg IL-1 Trap IP (n = 5), 1 mg/kg IL-1 Trap IP (n = 5), or 30 mg/kg IL-1 Trap IP (n = 5) to explore the efficacy over a previously tested wide range of doses[12] or matching volume of saline IP (n = 10) at the time of coronary ligation. Five ICR mice underwent sham operations. After initial dosing during ligation surgery, mice received additional doses every 48 hours until euthanasia.

Doppler Echocardiography

All animals underwent transthoracic echocardiography before surgery and 7 days after surgery. Doppler echocardiography was performed with the Vevo770 imaging system (VisualSonics Inc., Toronto, Ontario, Canada) and a 30-MHz probe. The transducer was positioned on the left anterior side of the chest. The heart was first imaged in the 2-dimensional mode in the short-axis view of the left ventricle. The M-mode cursor was positioned perpendicular to the anterior and posterior wall to measure left ventricular (LV) end-diastolic diameter (LVEDD), LV end-systolic diameters (LVESD), anterior wall diastolic thickness, anterior wall systolic thickness, posterior wall diastolic thickness, and posterior wall systolic thickness. According to the American Society of Echocardiography recommendations,[13] M-mode images were then obtained at the level of the papillary muscles below the mitral valve tip. Apical 4- and 5-chamber views were also obtained to measure transmitral flow, left ventricular outflow, and transaortic flow velocities. LV fractional shortening (FS) was calculated as follows: FS = (LVEDD – LVESD)/LVEDD × 100. Ejection fraction was calculated with the Teichholz formula. Transmitral and LV outflow tract pulsed Doppler flow spectra were obtained from the apical view. Measurement of the outflow tract flow was performed. Isovolumetric contraction (ICT), relaxation time (IRT), and ejection time (ET) were also measured to calculate the Tei index (Tei index = ICT + IRT/ET), which is associated with both systolic and diastolic dysfunction and worse outcomes in humans.[14] The number of akinetic segments was calculated based on a 16-segment map and the extent of damage was reported as a percentage of the LV. The investigator performing and reading the echocardiogram was blinded to the 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's trichrome (Sigma-Aldrich, St. Louis, MO). The areas of fibrosis and the whole LV were determined by computer morphometry using BIOQUANT imaging software (Nashville, TN). The ratio was used to compute scar area expressed as a percentage of the LV. The mean infarct wall thickness was calculated by averaging 3 equidistant regions of the scar. The minimal infarct thickness was also measured.

Apoptosis

The effects of IL-1 Trap on apoptosis were evaluated both ex vivo and in vitro. After euthanasia, hearts were extracted and fixed in formalin for at least 48 hours for the ex vivo measurements. A transverse section of the median third of the heart was obtained, included in paraffin, and cut into 5-μm slides. Cardiomyocyte apoptosis was defined by costaining for muscle actin (prediluted anti-mouse–sarcomeric actin antibody; Invitrogen, Carlsbad, CA) and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (DNA fragmentation, Apoptag, Chemicon, CA). Apoptosis was evaluated in the peri-infarct area of the LV, defined as the zone bordering the infarct where viable myocardium was prevalent.[15] The apoptotic rate was expressed as the number of apoptotic cardiomyocytes divided by the total number of cardiomyocytes per field at 400× under a light microscope.

In vitro apoptosis was evaluated in a primary mouse cardiomyocyte culture subjected to hypoxia/reoxygenation and the use of "ischemic" buffer to simulate ischemia–reperfusion. Ventricular cardiomyocytes were isolated using an enzymatic technique as previously described.[16] Briefly, mice were euthanized (100 mg/kg pentobarbital sodium IP) and the heart was quickly removed and placed in a Langendorff perfusion system for enzymatic digestion and cardiomyocyte isolation. The cardiomyocytes were cultured in the presence of 5% CO_2 for 1 hour in a humidified incubator at 37°C before the experimental protocol. Cardiomyocytes were then subjected to simulated ischemia for 40 minutes by replacing the cell medium with an "ischemia buffer" that contained 118 mM NaCl, 24 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.5 mM

$CaCl_2 \cdot 2H_2O$, 1.2 mM $MgCl_2$, 20 mM sodium lactate, 16 mM KCl, and 10 mM 2-deoxyglucose (pH adjusted to 6.2) at 37°C under hypoxic conditions (1% O_2 and 5% CO_2). After 40 minutes, reoxygenation was preformed for 18 hours by replacing the ischemic buffer with normal medium under normoxic conditions. At the same time, 0.25 mg/mL IL-1 Trap (n = 3) or an equivalent volume of normal medium (n = 3) was added to the plate. This IL-1 Trap concentration was extrapolated from previous experiments under similar conditions. After 18 hours of reoxygenation, the cells in two chamber slides were fixed by 4% formaldehyde/phosphate-buffered saline at 4°C for 25 minutes and cardiomyocyte apoptosis was analyzed using the same terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (DNA fragmentation, Apoptag). The stained cells were examined under an Olympus IX70 fluorescence microscope (Olympus, Center Valley, PA).

Statistical Analyses

We used analysis of variance for multiple comparisons with post hoc *t* test to explore between group differences. For comparisons of interval changes between multiple groups, we used random effects analysis of variance for repeated measures to determine the main effect of time, group, and time-by-group interaction. Statistical differences were considered significant if the *P* value was < 0.05. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Interleukin-1 Trap Reduces Interleukin-6 Response to Exogenous Interleukin-1β

Intraperitoneal injection of IL-1β led to a significant increase in IL-6 (4 hours) levels (421 ± 104 pg/mL) versus unstimulated mice (19 ± 7 pg/mL, $P = 0.020$, Fig. 1). As expected, pretreatment with 5 mg/kg IL-1 Trap prevented the increase in circulating IL-6 levels following IL-1β administration (15 ± 2 pg/mL, $P = 0.019$) versus IL-1 alone (Fig. 1).

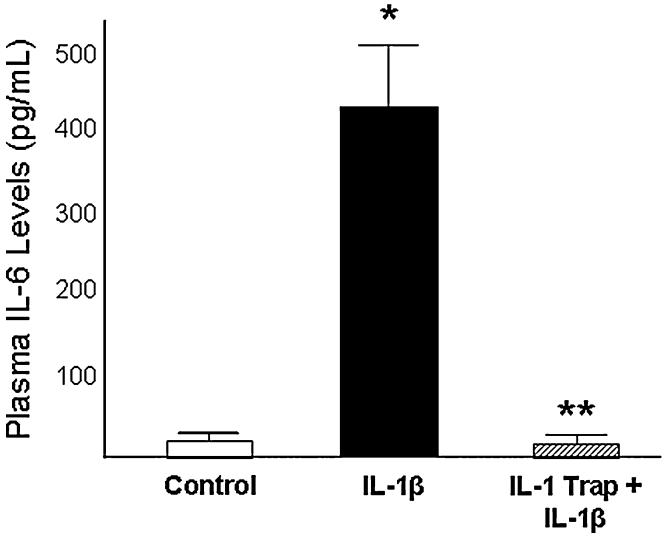

FIGURE 1. Interleukin (IL)-6 plasma levels after IL-1β stimulation. Pretreatment with 5 mg/kg IL-1 Trap blunted the IL-6 response at 4 hrs after IL-1β administration (100 ng [3 mg/kg]) ($P < 0.05$ versus control, $^{**}P < 0.05$ versus IL-1).

Interleukin-1 Trap Ameliorates Ventricular Remodeling After Acute Myocardial Infarction In Vivo

Baseline echocardiographic values were similar in all groups (Fig. 2). Significant increases in LV diameters (LVEDD and LVESD) and significant decreases in anterior wall diastolic thickness, anterior wall systolic thickness, and FS were observed 7 days after AMI in saline-treated mice ($P <$ 0.05 versus baseline and sham). Mice treated with 5 mg/kg and 30 mg/kg IL-1 Trap had significantly smaller increases in LVEDD and LVESD on Day 7 after AMI compared with saline-treated mice ($P < 0.05$ for all comparisons; Fig. 2). These doses of IL-1 Trap also lead to a smaller number of akinetic segments on Day 7 (2.0 ± 0.4 [5 mg/kg IL-1 Trap], 2.0 ± 0.5 [30 mg/kg IL-1 Trap] versus 3.7 ± 0.4 [saline], $P <$ 0.01 for each comparison). Moreover, mice treated with 5 mg/kg IL-1 Trap 5 had a lower Tei index at 7 days, reflecting better systolic and diastolic myocardial performance (0.19 ± 0.04 [IL-1 Trap] versus 0.45 ± 0.08 [saline], $P = 0.009$). The FS/Tei index, which correlates more closely with invasive measurements of +dP/dt, trended in favor of mice treated with 5 mg/kg IL-1 Trap (147% ± 10%) but failed to reach conventional statistical significance (68% ± 4%, $P = 0.062$ versus saline). The benefits of 5 mg/kg and 30 mg/kg IL-1 Trap were paralleled by smaller, nonsignificant improvements in mice receiving 1 mg/kg IL-1 Trap. The anterior (infarct) wall thickness was not significantly different between mice treated with saline (0.52 ± 0.02 mm), 1 mg/kg IL-1 Trap (0.48 ± 0.04 mm), 5 mg/kg IL-1 Trap (0.54 ± 0.05 mm), and 30 mg/kg IL-1 Trap (0.53 ± 0.06 mm, $P > 0.05$ for all comparisons).

Interleukin-1 Trap Does Not Affect Scar Formation

Treatment with IL-1 Trap did not significantly affect LV infarct scar formation (24.5% ± 6.5%) as measured by trichrome fibrosis staining at 7 days when compared with saline control-treated mice (32.8% ± 5.8%, $P = 0.57$). There was also no difference in mean infarct thickness (0.32 ± 0.08 mm versus 0.37 ± 0.11 mm, $P = 0.51$) or minimal infarct thickness (0.22 ± 0.08 mm versus 0.20 ± 0.13 mm, $P = 0.77$) at microscopy between mice treated with saline or IL-1 Trap, respectively.

Interleukin-1 Trap Inhibits Cardiomyocyte Apoptosis Ex Vivo and In Vitro

As expected, permanent coronary artery ligation increased the apoptotic rate of cardiomyocytes in the peri-infarct area (1.75% ± 0.25% [saline] versus 0.01% ± 0.01% [sham], $P < 0.001$). Treatment with 5 mg/kg IL-1 Trap significantly reduced the apoptotic rate in the peri-infarct myocardium (0.75% ± 0.25%, $P = 0.036$ versus saline; Fig. 3A).

In a primary culture of cardiomyocytes in vitro, 40 minutes of simulated ischemia and reoxygenation increased apoptotic nuclei from 3% ± 2% (nonischemic control) to

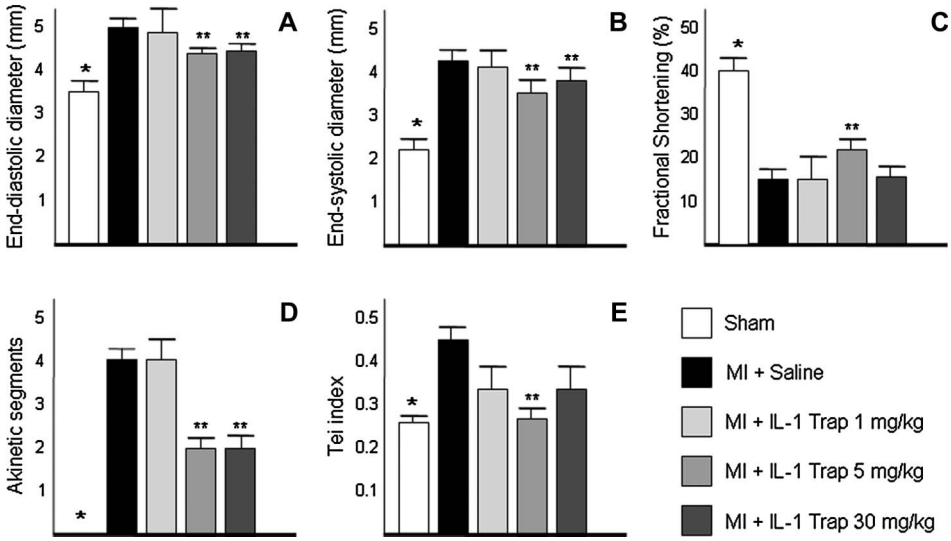

FIGURE 2. Echocardiographic measurements 7 days after coronary artery ligation surgery. Interleukin (IL)-1 Trap treatment attenuated changes in (A) left ventricular end diastolic and (B) systolic diameters and partially preserved (C) fractional shortening. IL-1 Trap also decreased the number of (D) akinetic segments and increased (E) Tei index (*$P < 0.05$ sham versus saline; **$P < 0.05$ treatment versus saline).

19% ± 1% of total cells ($P < 0.05$). Furthermore, incubation with IL-1 Trap (0.25 mg/mL) reduced apoptotic cells to 14% ± 2% ($P < 0.05$ versus ischemia/reoxygenation; Fig. 3B).

DISCUSSION

Acute myocardial infarction activates reparative processes that are initially beneficial but may produce pathologic changes over time. Prolonged ischemia leads to cell death and cardiac remodeling, characterized by progressive dilatation of the chambers, thinning of the infarct wall, and hypertrophy of viable segments.[17–19] Cardiomyocyte loss resulting from apoptosis plays a fundamental role in cardiac remodeling and progression toward heart failure.[5,20,21] IL-1 is the prototypal inflammatory cytokine and sits at the crossroads between apoptosis and inflammation.[5,6] Primarily produced by activated leukocytes, IL-1 further activates neutrophils, monocytes, platelets, endothelial cells, and cardiomyocytes in a paracrine and systemic fashion.[2,3,22] Ventricular myocytes may also directly contribute to this process through local production of IL-1 under ischemic conditions.[23,24]

In the current study, we show for the first time that IL-1 Trap, a synthetic protein that binds circulating IL-1 and prevents membrane receptor binding,[16] ameliorates pathologic cardiac remodeling after experimental AMI in mice. We also show that IL-1 Trap reduced cardiomyocyte apoptosis ex vivo after experimental AMI and in vitro in a primary cardiomyocyte model of ischemia/reperfusion. The beneficial effects of IL-1 Trap on cardiac remodeling, therefore, may be explained, at least in part, by a reduction in cardiomyocyte apoptosis.

IL-1 Trap is composed of the IL-1 binding domains of the IL-$1R_1$ and the IL-1 receptor accessory protein (IL-$1R_{AcP}$).[12] Incorporation of the IL-$1R_{AcP}$ domain increases binding affinity to free IL-1α and IL-1β above that obtained with the soluble receptor alone and markedly suppresses IL-1 signaling in both human and animal models.[12,25] The humanized version of IL-1 Trap, Rilonacept (Arcalyst; Regeneron, Tarrytown, NY), is approved by the Food and Drug Administration for the treatment of cryopyrin-associated periodic syndromes, a condition characterized by enhanced IL-1 activity.[25,26] Our data support the pathologic role of IL-1 in postinfarction cardiac remodeling and confirm that IL-1 Trap effectively blocks IL-1-induced effects in the mouse in vivo.

These data confirm previous reports describing the phenotype of IL-$1R_1^{-/-}$ mice that experienced reduced cardiac remodeling after AMI[9] and our own report describing the use of exogenous rhIL-1Ra to attenuate cardiomyocyte apoptosis and ventricular remodeling after AMI.[10] An earlier report, however, described worsened ventricular remodeling, reduced infarct healing, and increased ventricular in C57BL/6 mice after AMI after administration of a monoclonal hamster anti-mouse IL-1β antibody.[11] Although the mechanism accounting for these differences remains unclear, we speculate that use of a heterologous antibody (cross-species) may have contributed to the negative outcomes with the hamster anti-mouse IL-1β antibody. Alternatively, whereas rhIL-1Ra and IL-1 Trap provide blockade of both IL-α and IL-1β, targeted neutralization of IL-1β may have promoted unopposed IL-1α activity.[27,28]

Recombinant human IL-1Ra Anakinra - Kineret (Amgen Inc, Thousand Oaks, CA) is currently undergoing early-stage clinical investigation in both acute coronary syndromes and myocardial infarction.[29,30] However, anakinra has a relatively short half-life of 4 to 6 hours and therefore requires daily administration. Rilonacept has a substantially longer half-life that allows for once-weekly administration in patients with cryopyrin-associated periodic syndromes and may represent a preferable option for IL-1 blockade in clinical studies.

We tested an IL-1 Trap dose (5 mg/kg) known to reduce IL-1 signaling in previously published models.[12] We also explored two additional doses, 1 mg/kg and 30 mg/kg, on LV dimensions and function after simulated myocardial infarction. Although these experiments did not reveal significant differences between IL-1 Trap doses, treatment with 5 mg/kg was the only dose to produce statistically significant ameliorations of changes in LVESD and FS, two markers that are associated with improved outcomes after AMI in human patients.

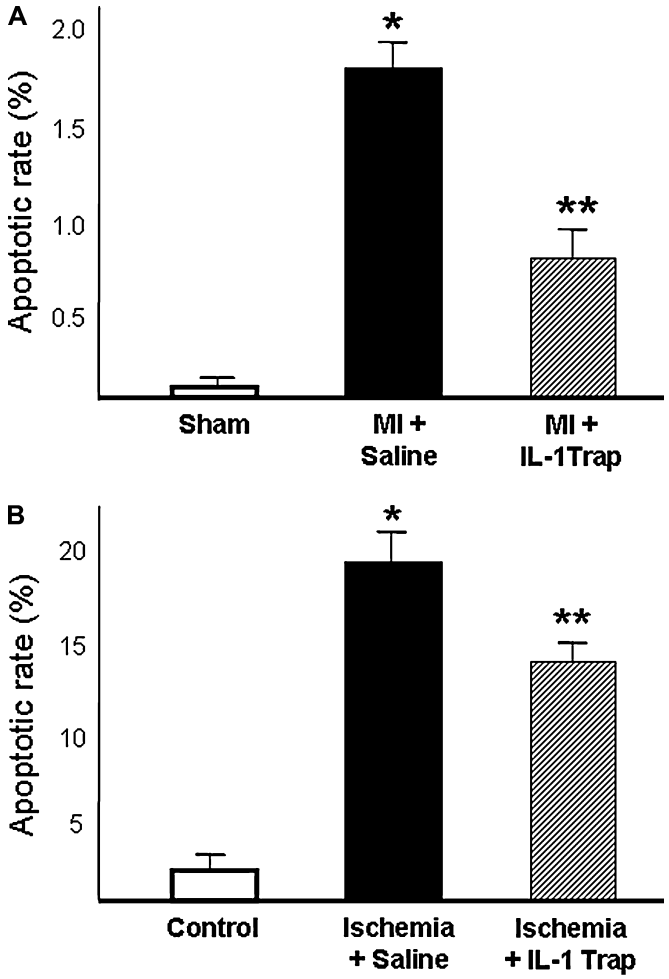

FIGURE 3. Apoptotic rate of cardiomyocytes. (A) Interleukin (IL)-1 Trap reduced the cardiomyocyte apoptotic rate in the peri-infarct area 14 days after coronary artery ligation surgery. (B) Simulated ischemia (18 hours) and reperfusion increased the number of apoptotic cultured cardiomyocytes. IL-1 Trap (0.25 mg/mL) at the time of reperfusion led to a significant reduction in apoptosis (*$P < 0.05$ versus sham/control; **$P < 0.05$ versus saline).

Previous murine studies of IL-1 Trap in experimental arthritis found no difference between anit-IL-1 activity of 10 mg/kg and 31 mg/kg.[12] Additional studies may be necessary to establish optimal dosing in human patients with AMI.

The apparent superiority of the 5-mg/kg dose may be attributable to the distinct pharmacokinetic/pharmacodynamic properties of cytokine binding proteins. Cytokine binding proteins with prolonged elimination half-lives often exhibit nontraditional dose–response curves that describe a maximum effective concentration. At concentrations below this maximum, increasing protein concentration results in increased cytokine binding and further inhibition of cytokine activity. At concentrations above this maximum, however, increasing protein concentration produces a "reservoir" effect whereby the reversible cytokine protein binding may prolong the effective half-life of the cytokine.[31]

We acknowledge multiple limitations to the current study such as a relatively small sample size, use of a single species and gender, and the use of operator-dependent echocardiography to assess left ventricular function. Our use of a permanent coronary ligation model may induce a different inflammatory response than the ischemia/reperfusion models[32] used in previous investigations of IL-$1R_1^{-/-}$ mice. The model of permanent coronary ligation constitutes a model of more severe damage leading to rapid LV enlargement, in which only a small rim of myocardium is preserved. We focused on the nonreperfused model because patients without reperfusion or with poor tissue level reperfusion despite recanalization of the epicardial coronary artery are those at highest risk of developing heart failure[33] and hence there is an urgent need for adjuvant treatments in these patients. While acknowledging limitations of the current study, we believe these data constitute a valuable validation of the benefits of IL-1 blockade in AMI and the translational potential of IL-1 Trap.

CONCLUSION

Myocardial infarction promotes cell death and cardiac remodeling leading to heart failure. IL-1 Trap is a novel IL-1 inhibitor that reduces cardiomyocyte apoptosis and ameliorates cardiac remodeling after experimental AMI in the mouse.

ACKNOWLEDGMENTS

We thank Drs. Charles A. Dinarello (University of Colorado) and Stanley Wiegand (Regeneron Pharmaceuticals Inc.) for their thoughtful review of the manuscript.

REFERENCES

1. National Heart, Lung, and Blood Institute. *Morbidity and Mortality: 2007 Chart Book on Cardiovascular and Lung Diseases*. Bethesda, MD: National Institutes of Health; 2007.
2. Herskowitz A, Choi S, Ansari A, et al. Cytokine mRNA expression in postischemic/reperfused myocardium. *Am J Pathol*. 1995;146:419–428.
3. Dewald O, Ren G, Duerr GH, et al. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. *Am J Pathol*. 2004;164:665–677.
4. Testa M, Yeh M, Lee P, et al. Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. *J Am Coll Cardiol*. 1996;28:964–971.
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. Dinarello CA. Interleukin-1. *Cytokine Growth Factor Rev*. 1997;8:253–265.
7. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448–454.
8. Di Iorio A, Ferrucci L, Sparvieri E, et al. Serum IL-1beta levels in health and disease: a population-based study. The InCHIANTI study. *Cytokine*. 2003;22:198–205.
9. 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.
10. 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.
11. Hwang MW, Matsumori A, Furukawa Y, et al. Neutralization of interleukin-1 in the acute phase of myocardial infarction promotes the progression of left ventricular remodeling. *J Am Coll Cardiol*. 2001;38:1546–1553.

12. Economides AN, Carpenter LR, Rudge JS, et al. Cytokine traps: multicomponent, high-affinity blockers of cytokine action. *Nat Med*. 2003;9: 47–52.
13. Schiller NB, Shah PM, Crawford M, et al; for the American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography: American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr*. 1989;2:358–367.
14. Tei C, Ling LH, Hodge DO, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function: a study in normal and dilated cardiomyopathy. *Am J Cardiol*. 1995;26:357–366.
15. Abbate A, Bussani R, Biondi-Zoccai GG, et al. Infarct-related artery occlusion, tissue markers of ischaemia, and increased apoptosis in the peri-infarct viable myocardium. *Eur Heart J*. 2005;26:2039–2045.
16. Das A, Xi L, Kukreja RC. Phosphodiesterase-5 inhibitor sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis: essential role of nitric oxide signaling. *J Biol Chem*. 2005;280:12944–12955.
17. Cohn JN, Ferrari R, Sharpe N; International Forum on Cardiac Remodeling. Cardiac Remodeling—concepts and clinical implications: a consensus paper from international forum on cardiac remodeling. *J Am Coll Cardiol*. 2000;35:569–582.
18. Nian M, Lee P, Khaper N, et al. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res*. 2004;94:1543–1553.
19. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction—experimental observations and clinical implications. *Circulation*. 1990;81:1161–1172.
20. Sam F, Sawyer DB, Chang DL, et al. Progressive left ventricular remodeling and apoptosis late after myocardial infarction in mouse heart. *Am J Physiol Heart Circ Physiol*. 2000;279:H422–H428.
21. MacLellan WR, Schenider MD. Death by design. Programmed cell death in cardiovascular biology and disease. *Circ Res*. 1997;81:137–144.
22. Guillen I, Blanes M, Gomez-Lechon MJ, et al. Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. *Am J Physiol*. 1995;269:R229–R235.
23. Carlson DL, Maass DL, White J, et al. Caspase inhibition reduces cardiac myocyte dyshomeostasis and improves cardiac contractile function after major burn injury. *J Appl Physiol*. 2007;103:323–330.
24. Maass DL, White J, Horton JW. Nitric oxide donors alter cardiomyocyte cytokine secretion and cardiac function. *Crit Care Med*. 2005;33: 2794–2803.
25. Church LD, McDermott MF. Rilonacept in cryopyrin-associated periodic syndromes: the beginning of longer-acting interleukin-1 antagonism. *Nat Clin Pract Rheumatol*. 2009;5:14–15.
26. Available at: www.regeneron.com/il_1trap.html. Accessed July 23, 2009.
27. Zell R, Geck P, Werdan K, et al. TNF-α and IL-1α inhibit both pyruvate dehydrogenase activity and mitochondrial function in cardiomyocytes: evidence for primary impairment of mitochondrial function. *Moll Cell Biochem*. 1997;177:61–67.
28. Isoda K, Kamezawa Y, Tada N, et al. Myocardial hypertrophy in transgenic mice overexpressing human interleukin 1α. *J Card Failure*. 2001;7: 355–364.
29. Crossman DC, Morton AC, Gunn JP, et al. Investigation of the effect of interleukin-1 receptor antagonist (IL-1ra) on markers of inflammation in non–ST elevation acute coronary syndromes (The MRC-ILA-HEART Study). *Trials*. 2008;9:8.
30. Available at: www.clinicaltrials.gov/ct2/show/NCT00789724. Accessed September 14, 2009.
31. Klein B, Brailly H. Cytokine-binding proteins: stimulating antagonists. *Immunol Today*. 1995;16:216–220.
32. 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.
33. Abbate A, Kontos MC, Biondi-Zoccai GGL. No-reflow: the next challenge in treatment of ST-elevation acute myocardial infarction. *Eur Heart J*. 2008;29:1795–1797.