**UltraRapid Communication**

**IL-10 Inhibits Inflammation and Attenuates Left Ventricular Remodeling After Myocardial Infarction via Activation of STAT3 and Suppression of HuR**

Prasanna Krishnamurthy, Johnson Rajasingh, Erin Lambers, Gangjian Qin, Douglas W. Losordo, Raj Kishore

**Abstract**—Persistent inflammatory response has adverse effects on left ventricular (LV) function and remodeling following acute myocardial infarction. We hypothesized that suppression of inflammation with interleukin (IL)-10 treatment attenuates LV dysfunction and remodeling after acute myocardial infarction. After the induction of acute myocardial infarction, mice were treated with either saline or recombinant IL-10, and inflammatory response and LV functional and structural remodeling changes were evaluated. IL-10 significantly suppressed infiltration of inflammatory cells and expression of proinflammatory cytokines in the myocardium. These changes were associated with IL-10–mediated inhibition of p38 mitogen-activated protein kinase activation and repression of the cytokine mRNA–stabilizing protein HuR. IL-10 treatment significantly improved LV functions, reduced infarct size, and attenuated infarct wall thinning. Myocardial infarction–induced increase in matrix metalloproteinase (MMP)-9 expression and activity was associated with increased fibrosis, whereas IL-10 treatment reduced both MMP-9 activity and fibrosis. Small interfering RNA knockdown of HuR mimicked IL-10–mediated reduction in MMP-9 expression and activity in NIH3T3 cells. Moreover, IL-10 treatment significantly increased capillary density in the infarcted myocardium which was associated with enhanced STAT3 phosphorylation. Taken together, our studies demonstrate that IL-10 suppresses inflammatory response and contributes to improved LV function and remodeling by inhibiting fibrosis via suppression of HuR/MMP-9 and by enhancing capillary density through activation of STAT3. (Circ Res. 2009;104:e9–e18.)

**Key Words:** IL-10 ■ myocardial infarction ■ cytokines ■ inflammation ■ cardiac remodeling

Myocardial infarction (MI) is among the leading causes of death in Western societies and often results in the development of heart failure. Myocardial infarct size (acute phase), followed by adverse left ventricular (LV) remodeling (dilation and fibrosis) and cardiac dysfunction, are major determinants in the pathogenesis of cardiac diseases. A number of cardiac pathophysiological conditions including MI and ischemia/reperfusion injury, leading to heart failure are associated with activation of inflammatory mediators in the heart.

Expression of proinflammatory cytokines (eg, tumor necrosis factor [TNF]-α, interleukin [IL]-1, and IL-6) and the antiinflammatory cytokine IL-10 mediate homeostasis within the heart in response to injury. However, a sustained expression of proinflammatory mediators at sufficiently high concentrations could lead to adverse outcome in the failing heart. Increased matrix metalloproteinase (MMPs) production associated with sustained inflammatory response may lead to excessive extracellular matrix (ECM) degradation in the early phase of MI, impairing infarct healing and aggravating early remodeling which in turn causes cardiac rupture. Also, the increased cytokine gene expression during acute phase of inflammation evokes a secondary, self-sustaining autocrine and paracrine growth factor and cytokine expression.

IL-10, a potent antiinflammatory cytokine, is a strong deactivator of monocytes and suppressor of various proinflammatory mediators. However, the therapeutic effect of systemic IL-10 on inflammation mediated LV dysfunction and remodeling, and the molecular signaling that governs these effects remains to be studied.

We have reported previously that IL-10, a potent antiinflammatory cytokine, inhibits a panel of proinflammatory cytokine/chemokine mRNA expression in both human monocytic cell line (U937) and primary mouse macrophages via inhibiting the cytokine mRNA–stabilizing protein HuR and suppression of p38 mitogen-activated protein kinase (MAPK). p38 has also been implicated in oxidant stress–induced cardiac dysfunction and remodeling. Ischemic oxidative stress of cardiomyocytes stimulate STAT3 (signal transducer and ac-
tivator of transcription 3) exerts cardioprotection in the ischemic heart. However, the potential role of IL-10–mediated antiinflammatory responses and modulations in either STAT3 or HuR signaling during post-MI remodeling has not been explored. This study was undertaken to elucidate the role of IL-10 in modulating inflammation, LV dysfunction and remodeling, and the signaling mechanisms that regulate IL-10–mediated effects on remodeling.

Materials and Methods

Vertebrate Animals

All experiments conformed to the protocols approved by the Institutional Animal Care and Use Committee. Nine-week-old mice of C57BL/6J background were procured from The Jackson Research Laboratory (Bar Harbor, Me). The mice were allowed to acclimate for 10 days under standard animal care conditions.

Antibodies and Reagents

Antibodies against phospho (p)–p38, p–STAT3, β-actin, and total p38 were purchased from Cell Signaling Inc (Boston, Mass); anti–HuR antibodies were from Upstate Inc (Lake Placid, NY); p–serine from Anaspec Inc (San Jose, Calif); total-STAT3 was from Santa Cruz Biotechnology Inc (Santa Cruz, Calif). Recombinant murine and human IL-10 and TNF-α were obtained from R&D Systems (Minneapolis, Minn). Limnoplysaccharide was obtained from Sigma Aldrich Inc (St Louis, Mo), and cucurbitacin I was from Calbiochem (San Diego, Calif). Specific mouse HuR small interfering (si)RNA and a scrambled control siRNA were purchased from Ambion Inc (Austin, Tex).

Cell Culture and Antibodies

NIH3T3 Swiss albino fibroblasts (American Type Culture Collection) were cultured in DMEM (Mediatech, Herndon, Va) with 10% FCS, 1-glutamine, and 0.1 mmol/L β-mercaptoethanol. Human umbilical vein endothelial cells (HUVECs) were cultured in EBM-2 media (Clonitech, Palo Alto, Calif) with 10% FCS. Cells were stimulated with lipopolysaccharide, TNF-α, and/or IL-10 at 10 ng/mL and cucurbitacin at 1 μmol/L, unless otherwise indicated.

MI and Study Design

Mice were subjected to MI by ligation of left anterior descending coronary artery as described previously. These mice were injected subcutaneously with mouse recombinant IL-10 (50 μg/kg body weight; MI + IL-10 group) or saline (MI group) on 0, 1, 3, 5 and 7 days post-MI. The mice in the sham group underwent the same procedure except for the left anterior descending coronary artery ligation. These mice received saline subcutaneously. Inflammatory response was assessed after 3 days, LV functional changes were assessed on 14 and 28 days, and structural remodeling was assessed at 28 days post-MI.

Echocardiography

Transthoracic 2D M-mode echocardiogram was obtained using Vevo 770 (VisualSonics, Toronto, Canada) equipped with a 30-MHz transducer. Echocardiographic studies were performed before (baseline) and at 14 and 28 days post-MI on mice anesthetized with a mixture of 1.5% isoflurane and oxygen (1 L/min). M-mode tracings were used to measure LV wall thickness, LV end-systolic diameter (LVESD), and LV end-diastolic diameter (LVEDD). The mean value of 9 measurements was determined for each sample. Percentage of fractional shortening (%FS) and ejection fraction (EF%) was calculated as described.

Morphometric Studies

The hearts were perfusion-fixed with 10% buffered formalin. Hearts were cut into 3 slices (apex, mid-LV and base) and paraffin-embedded. The morphometric analysis, including infarct size, wall thickness, and percentage of fibrosis, was performed on Masson’s trichrome stained tissue sections using ImageJ software (NIH, version 1.30; http://rsb.info.nih.gov/ij). Wall thickness was measured perpendicular to the infarcted wall at 3 separate regions and averaged. Fibrosis area and total LV area were measured and expressed as percentage of fibrosis.

Immunohistochemistry

Immunohistochemistry for tissue sections was performed as described previously. Deparaffinized tissue sections were permeabilized and stained with anti–CD68 (Serotec, Raleigh, NC) for inflammatory cell infiltration, anti–CD31 antibody (BD Biosciences, San Jose, CA) for capillary density, and α-smooth muscle actin (α-SMA) (Sigma Aldrich) for arteriole smooth muscle (SM) staining, followed by incubation with respective secondary antibodies. Staining without the primary antibodies was used as control for nonspecific fluorescence. Nuclei were counterstained with 4’,6-diamidino-2-phenylindole (DAPI) (1:5000, Sigma Aldrich), and sections were examined with a fluorescent microscope (Nikon ECLIPSE TE200). Capillary density (CD31-positive) and inflammatory cell infiltration (CD68-positive) were assessed at 10 randomly selected high visual fields in the border zone of infarcted myocardium and expressed as number per high-power visual field. Arterioles were defined as round-shaped, α-SMA-positive vessels with diameters of >10 μm (small arterioles) to >20 μm (large arterioles).

TUNEL Staining

TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling) staining was carried out on 4-μm thick paraffin-embedded sections according to the instructions of the manufacturer (Cell Death Detection Assay; Roche, Indianapolis, Ind). Cardiac myocytes were identified using α-sarcomeric actinin antibodies (Sigma Chemicals). DAPI staining was used to count the total number of nuclei. The index of apoptosis was calculated as the percentage of apoptotic myocyte nuclei per total number of nuclei.

Western Blot Analysis

Tissue lysates were prepared from the LV infarct border zone using ice-cold radio immunoprecipitation assay buffer (158 mmol/L NaCl, 10 mmol/L Tris HCl, pH 7.2, 1 mmol/L EGTA, 1 mmol/L sodium orthovanadate, 0.1% sodium dodecyl sulfate, 1.0% Triton X-100, 1% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride). Proteins (50 μg) were electrophoresed and analyzed using anti-phospho (p)-p38, anti–HuR, and anti–p-serine (p-ser) (immunoprecipitated with STAT3) antibodies. Equal protein loading in each lane was verified using antibodies against the corresponding total protein or β-actin.

Quantitative RT-PCR

Gene expression levels of TNF-α, IL-1β, IL-6, monocyte chemotractant protein (MCP)-1, interferon-γ inducible protein (IP)-10, TNF receptor (TNFR1), TNFR2, MMP-9, MMP-2, and vascular endothelial growth factor (VEGF)-A were quantified in the border zone of infarct as described previously. RNA was collected from heart tissue, NIH3T3 cells, and HUVECs with RNA STAT-60 (TEL-TEST Inc, Friendswood, Tex). Total RNA was reverse-transcribed with iScript CDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, Calif), and amplification was performed using TaqMan 7300 (Applied Biosystems, Foster City, Calif). Relative mRNA expression of target genes was normalized to the endogenous 18S control gene (Applied Biosystems).

In-Gel Zymography

MMP activity in LV tissue lysate or cell culture media was measured using gelatin in-gel zymography. After staining with Coomassie blue and destaining, clear and digested regions representing MMP activity were quantified by densitometry using a Kodak 2000 documentation system.
Statistical Analyses

Data are presented as means±SE. Data were analyzed using Student’s t tests or 1-way ANOVA and a post hoc Tukey’s test. Probability values of <0.05 were considered to be significant.

Results

IL-10 Treatment Suppresses Inflammation in the Myocardium at Three Days Post-MI

Immunohistochemical staining of CD68-positive cells (CD68⁺ cells) on cardiac tissue sections was carried out to study the inflammatory cell infiltration on 3 days post-MI. Infiltration of CD68⁺ cells (macrophage and monocyte) in the border zone of LV infarct increased at 3 days after MI (P<0.01 versus sham; Figure 1). IL-10 treatment significantly inhibited CD68⁺ cells infiltration at the injury site (P<0.01 versus MI group; Figure 1).

mRNA expression of various proinflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α, MCP-1, IP-10) in the myocardium at 3 days post-MI was assessed by quantitative RT-PCR. Increased inflammatory cell infiltration was associated with increased mRNA expression of proinflammatory cytokines and chemokines in the myocardium (P<0.01 versus sham; Figure 2). IL-10 treated group showed significant reduction in the mRNA expression levels (P<0.05 versus MI; Figure 2).

Stimulation of inflammatory responses of TNF-α is mediated through TNF receptors (TNFR1 and TNFR2). Ablation of TNFR1 blunts heart failure and improves survival, whereas ablation of the TNFR2 gene exacerbates heart failure and reduces survival. Quantitative RT-PCR analyses showed increased mRNA expression of TNFR1 in the myocardium at 3 days post-MI (P<0.01 versus sham; Figure 2). Interestingly, IL-10 treatment significantly reduced TNFR1 levels in the myocardium (P<0.01 versus MI; Figure 2). However, mRNA expression of TNFR2 increased after MI (fold change; MI, 2.53±0.12; MI+IL-10, 2.04±0.21; P<0.05 versus sham), but no significant difference was observed between the MI groups.

IL-10 Attenuates Post-MI Left Ventricular Dysfunction, Reduces Infarct Size, and Attenuates Infarct Wall Thinning

LV function was assessed by echocardiography. M-mode tracings were analyzed at 14 and 28 days post-MI. Similar changes were observed at these 2 time points. MI increased LVESD and LVEDD (P<0.01 versus baseline; Figure 3A and 3B and the Table) and decreased %FS and percentage of ejection fraction (%EF) at 28 days post-MI (P<0.05 versus baseline; Figure 3B and the Table). IL-10 treatment attenuated cardiac dysfunction with significantly lowered LVESD and LVEDD and increased %FS and %EF as compared to MI group (P<0.05 versus MI group; Figure 3B). Heart rates were not significantly different among the groups. Similar trends were observed at 14 days post-MI (Table). Infarct size was measured as percentage of the LV circumference from trichrome stained sections at 28 days post-MI. Infarct size of 43.13±0.65% was observed in MI group (Figure 4A). IL-10 treatment resulted in a significant reduction in the infarct size (30.86±1.24%, P<0.01 versus MI group; Figure 4A). Interestingly, IL-10 treatment showed an increase in the infarct wall thickness (0.33±0.01 mm; P<0.01 versus MI; Figure 4B) as compared to a thin wall in MI group (0.19±0.02 mm; Figure 4B).

Figure 1. A, Immunofluorescent staining of inflammatory cells (CD68-positive, green fluorescence) in the heart at 3 days post-MI. Bar graph shows quantitative analysis of infiltrating CD68⁺ cells at 3 days post-MI. IL-10 inhibited CD68⁺ cells infiltration as compared to MI and sham hearts. *P<0.01 vs sham, #P<0.01 vs MI.

Figure 2. Quantitative analysis of mRNA expression of proinflammatory cytokines, chemokines, and TNF-α receptor (TNFR) in the border zone of LV infarct at 3 days post-MI. mRNA expression normalized to 18S expression and depicted as fold change vs sham. *P<0.01 vs sham; #P<0.05 vs MI.
IL-10 Attenuates MI-Induced Cardiac Cell Death by Apoptosis
Heart section was stained with the TUNEL method to detect cardiac apoptosis at 3 and 28 days post-MI. A similar trend was observed at both the time points after MI. MI increased the number of apoptotic cells in the border zone of infarction as compared to sham group (percentage of apoptosis; MI, 3.07 ± 0.24; sham, 0.32 ± 0.01; P < 0.01 versus sham; Figure 4C). However, IL-10 treatment significantly reduced the number of apoptotic cells in the border zone of LV infarct (percentage of apoptosis; MI IL-10, 1.83 ± 0.28; P < 0.01 versus MI; Figure 4C). To determine the apoptotic cardiomyocytes, the sections were stained for α-sarcomeric actinin. The number of apoptotic myocytes increased after 28 days post-MI as compared to the sham group (percentage of apoptosis; sham, 0.34 ± 0.02; MI, 1.67 ± 0.11; P < 0.01 versus sham). However, the myocyte apoptosis was not significantly different between the 2 MI groups (MI IL-10, 1.56 ± 0.07; P < 0.01 versus sham). Whereas, it is interesting to note that there was increased number of TUNEL-positive cells lining the capillaries after MI, which was significantly lower in mice treated with IL-10 (P Krishnamurthy, J Rajasingh, E Lambers, G Qin, DW Losordo, R Kishore, unpublished data, 2008).

IL-10 Suppresses Phosphorylation of p38 MAPK and the mRNA-Stabilizing Protein HuR and Activates STAT3 in the Myocardium Post-MI
To investigate intracellular signaling pathways that are affected by IL-10 treatment, we measured the protein levels of the cytokine mRNA–stabilizing protein HuR and activation

Table. Echocardiographic Measurements

<table>
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<tr>
<th>Parameters</th>
<th>Baseline (n=10)</th>
<th>MI (n=8)</th>
<th>MI+IL-10 (n=8)</th>
<th>MI (n=8)</th>
<th>MI+IL-10 (n=8)</th>
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<tr>
<td>LVEDD, mm</td>
<td>4.15±0.11</td>
<td>5.72±0.31*</td>
<td>4.92±0.19†</td>
<td>6.26±0.20</td>
<td>5.11±0.04#</td>
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<td>LVESD, mm</td>
<td>2.99±0.08</td>
<td>5.31±0.28*</td>
<td>3.85±0.24†</td>
<td>5.79±0.17</td>
<td>4.08±0.20#</td>
<td>*&lt;0.01; †&lt;0.01; #&lt;0.01</td>
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<td>EF, %</td>
<td>54.10±1.98</td>
<td>15.02±3.34*</td>
<td>44.77±3.52†</td>
<td>16.07±2.74</td>
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<tr>
<td>FS, %</td>
<td>27.83±1.29</td>
<td>6.98±1.63*</td>
<td>22.52±2.02†</td>
<td>7.48±1.33</td>
<td>19.61±1.20#</td>
<td>*&lt;0.05; †&lt;0.01; #&lt;0.01</td>
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<td>Heart rate, bpm</td>
<td>424±7.93</td>
<td>456±16.72</td>
<td>447±12.25</td>
<td>480±21.67</td>
<td>480±10.69</td>
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Values are means±SE. bpm indicates beats per minute; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter. *Comparison between baseline and MI groups; †comparison between MI groups at 14 days post-MI; #comparison between MI groups at 28 days post-MI.
of p38 MAPKs at 3 days and STAT3 at 3 and 28 days in the LV after MI. Western blot analysis demonstrated that HuR protein expression was significantly upregulated after 3 days of MI (P<0.01 versus sham; Figure 5A). However, IL-10 significantly suppressed the protein expression of HuR (P<0.01; Figure 5A). Phosphorylation (activation) of p38 MAPK (p-p38) was increased in the myocardium at 3 days post-MI (P<0.01 versus sham; Figure 5B). However, IL-10 treatment significantly reduced the phosphorylation of p38 MAPK (P<0.01 versus MI; Figure 5B). To study the effect of IL-10 on STAT3, tissue lysate was immunoprecipitated with STAT3 antibodies and Western blot performed using anti–p-ser antibodies. MI increased phosphorylation of STAT3 (p-ser) at 28 days (P<0.01 versus sham; Figure 5C). Interestingly, STAT3 phosphorylation (p-ser) was further higher in myocardium of IL-10–treated mice after MI (P<0.05 versus MI; Figure 5C). Similar results were observed at 3 days post-MI.

IL-10 Treatment Enhances Capillary Density and Attenuates Arteriole SM Hyperplasia in the LV at Twenty-Eight Days Post-MI

Immunohistochemical staining of CD31-positive cells (CD31+) was carried out to assess the neovascularization in border zone of infarct and remote noninfarct area at 28 days post-MI. Capillary density (CD31+ cells) in the border zone increased in the myocardium at 28 days post-MI (P<0.01 versus sham; Figure 6A). Interestingly, IL-10 treatment further increased capillary density when compared to MI group (P<0.05 versus MI; Figure 6A). However, capillary density in the remote area was not different between the MI groups (data not shown). A previous report from our laboratory has shown that IL-10 significantly attenuated inflammation-mediated arterial SM hyperplasia in a murine carotid artery injury model.10 To assess the effect of IL-10 on arterioles in the LV after MI, tissue sections were stained with anti–α-SMA and the vessels >20 μm (large arteriole) were analyzed. It was interesting to note that the large arterioles were thicker with many layers of SM cells as compared to sham group indicating arterial hyperplasia following MI (P<0.01 versus sham; Figure 6B). However, IL-10 treatment attenuated SMC hyperplasia as compared to MI group (P<0.01 versus MI; Figure 6B). To determine the role of VEGF in IL-10–mediated enhancement of angiogenesis, quantitative RT-PCR analysis of the border zone of LV tissue at 28 days post-MI revealed increased VEGF-A expression, which was further higher in IL-10–treated group (P<0.05 versus MI; Figure 7A).

IL-10–Induced Upregulation of VEGF-A Is Mediated Through STAT3 in HUVECs

STAT3 is required for myocardial capillary growth and heart protection from ischemic injury.14 To determine the signaling mechanism involved in IL-10–mediated induction of VEGF, passage 4 HUVECs were grown until confluent and pretreated with cucurbitacin I (stat3 inhibitor, 1 μmol/L) for 1 hour. The cells were further treated with IL-10 or saline for another 6 hours. RNA extracted from treated cells was
subjected to quantitative RT-PCR analysis of VEGF-A gene expression. IL-10 treatment significantly increased VEGF-A expression ($P < 0.01$ versus control cells; Figure 7B). However, inhibition of STAT3 with cucurbitacin I significantly suppressed IL-10–induced VEGF-A gene expression ($P < 0.01$ versus IL-10–treated cells; Figure 7B), therefore suggesting that IL-10–induced VEGF-A is mediated through STAT3.

IL-10–Mediated Attenuation of LV Fibrosis Is Associated With Suppressed mRNA Expression and Activity of MMP-9 in the LV Post-MI

Quantitative analysis of trichrome-stained sections indicated increased fibrosis in the LV after 28 days post-MI as compared to sham ($P < 0.01$ versus sham; Figure 8A). Interestingly, we observed significantly reduced fibrosis in the LV following IL-10 treatment ($P < 0.05$ versus MI; Figure 8A).

MMPs play an important role in ECM remodeling following MI. MMP (MMP-2 and -9) expression, and activity increases rapidly in the heart post-MI. Here, we examined mRNA expression by quantitative RT-PCR (Figure 8B) and MMP activity by in-gel zymography (Figure 8C) in the border zone at 28 days post-MI. Similar trend was observed at 3 and 28 days post-MI. Quantitative RT-PCR analysis indicated that the mRNA expression of MMP-9 increased after MI ($P < 0.01$, MI versus sham; Figure 8B). However, IL-10 treatment significantly reduced MMP-9 mRNA expression levels in the LV ($P < 0.01$ versus MI; Figure 8B). However, mRNA expression and activity of MMP-2 was not significantly different between the MI groups at 3 and 28 days post-MI (P Krishnamurthy, J Rajasingh, E Lambers, G Qin, DW Losordo, R Kishore, unpublished data, 2008). Analysis of MMP activity using gelatin in-gel zymography (Figure 8C) indicated increased MMP-9 activity after MI ($P < 0.01$ versus sham; Figure 8C). IL-10 treatment showed reduced MMP-9 activity in the LV ($P < 0.01$; Figure 8C).
Figure 9A, IL-10 treatment significantly inhibited TNF-α–induced MMP-9 activity. IL-10 significantly inhibited TNF-α–induced MMP-9 gene expression (P<0.01 versus TNF-α–treated cells; Figure 9B) and activity (P<0.05 versus TNF-α–treated cells; Figure 9C). Interestingly, knockdown of HuR led to inhibition of TNF-α–induced MMP-9 gene expression (P<0.01, HuR-siRNA+TNF-α versus scrambled siRNA+TNF-α; Figure 9B) and activity (P<0.05, HuR-siRNA+TNF-α versus scrambled siRNA+TNF-α; Figure 9C), therefore mimicking IL-10 effects on MMP-9.

Discussion

Cardiac pathophysiological conditions like MI and reperfusion injury have been associated with the activation of proinflammatory cytokines such as IL-1β, TNF-α, and IL-6.24 Various reports have shown that strategies targeting inflammatory response in the heart had promising results with attenuation of cardiac remodeling.13,25–28 Here, we inhibited inflammatory response in the heart with mouse recombinant IL-10, a potent antiinflammatory cytokine, and studied LV function and remodeling. The important findings of this study are that IL-10 administration attenuated myocardial inflammation and MMP-9 (expression and activity) at 3 days after MI followed by attenuation of LV dysfunction and remodeling with effects on fibrosis and capillary density at 28 days post-MI. The above effects were suggested to be partly attributable to IL-10–mediated suppression of the cytokine mRNA–stabilizing protein HuR and STAT3 activation.

Inflammatory mediators have been implicated in LV remodeling, including cardiomyocyte hypertrophy,29 with alterations in fetal gene expression and contractile abnormalities.7,26,27 Our study has shown that the infiltration of CD68-positive monocytic/macrophages in the border zone of the myocardium was increased at 3 days post-MI. Inflammatory cell infiltration was associated with an increase in mRNA expression of various proinflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α, IP-10, MCP-1) after MI. These “stress-activated” cytokines are produced by various cell types in the myocardium, including cardiomyocytes or by the infiltrating inflammatory cells.

IL-10, a potent antiinflammatory cytokine, has been shown to limit the infiltration of inflammatory cells in vascular injury model.10 In agreement with the above study, IL-10 treatment inhibited inflammatory cells infiltration and proinflammatory cytokines and chemokines (P<0.05 versus MI) in the myocardium. The failure of anti–TNF-α therapy in humans using antibody was attributed partly to unaffected IL-6, IL-1β, and MCP-1 levels within the myocardium.30 Moreover, IL-1β has also been shown to have similar effects as that of TNF-α.27 An important finding of the present study is that IL-10 not only inhibits TNF-α but also other proinflammatory cytokines, which are shown to have adverse cardiac remodeling effects. In addition, IL-10 may attenuate remodeling through direct effects on protease activity without involvement of other cytokines.
Myocardial expression of cytokines contributes to depression of contractile performance and adverse LV remodeling.7,24 In the present study, echocardiography showed increase in LVEDD and LVESD and decrease in %FS and EF% after MI and IL-10 attenuated these effects at 28 days post-MI (P<0.05 versus MI). Cytokine-induced depression of contractile performance was suggested to be a result of interference with myocardial calcium handling.31,32 However, whether IL-10 affects calcium-mediated cardiac contractility is not known from the present study. The significant upregulation of proinflammatory cytokines (at 3 days) could trigger a second phase of elevated cytokines levels in the noninfarcted myocardium that promotes interstitial fibrosis and collagen deposition, leading to ventricular dysfunction.24 Our findings corroborated well with the above changes. Most importantly a recent study33 has shown that transplantation of bone marrow mononuclear cells (BM-MNCs) in infarcted mouse hearts led to a significant improvement in cardiac function. These BM-MNCs secreted significant amounts of IL-10, and the cardiac protection was associated with decreased T-lymphocyte accumulation, reactive hypertrophy, and myocardial collagen deposition. Also, various studies have reported that activation of MMPs and p38, and reduced angiogenesis and STAT3 have affected remodeling and cardiac dysfunction.6,12-14

Homeostasis of ECM (degradation and accumulation) play an important role in the pathogenesis of LV remodeling, which is controlled by MMPs and their tissue inhibitors (TIMPs).34,35 Increased MMPs production associated with sustained inflammatory response may lead to excessive ECM degradation, leading to cardiac rupture (in the acute phase) and adverse remodeling changes later.5-7

Our study shows that MI increased fibrosis (P<0.01 versus sham) associated with increased mRNA expression and gelatinolytic activity of MMP-9 in the LV (P<0.01 versus sham). These findings corroborate well with the previous finding, where both IL-1β and TNF-α have been associated with increased MMPs and fibrosis in the heart.29 Interesting finding of the present study is that IL-10 treatment significantly reversed these effects. However, MMP-2 expression and activity was not affected between the groups. IL-10–mediated MMP-9 suppression in the LV was also associated with inhibition of the mRNA-stabilizing protein HuR. Because MMP-9 expression in mesangial cells is mediated by the mRNA-stabilizing factor HuR,23 we examined the role of HuR in TNF-α–mediated MMP-9 induction in NIH3T3 (mouse fibroblast) cell line. Interestingly, siRNA-mediated HuR knockdown resulted in the suppression of TNF-α–mediated MMP-9 expression and activity. This suggests that IL-10–mediated HuR suppression could have led to MMP-9 inhibition. TNF-α augments IL-1β–stimulated release of MMP-9 but not MMP-2.26 Earlier reports have shown that targeted deletion of MMP attenuates early infarct rupture and cardiac contractility and remodeling in mice post-MI.6 In vitro experiments demonstrated that isolated canine mononuclear cells with neutralizing antibody to IL-10 inhibited TIMP-1 mRNA expression, which suggested that IL-10 may have a significant role in healing by inducing TIMP-1 expression.8 These findings along with previous reports suggest that IL-10 attenuation of LV remodeling might partly be attributable to reduced fibrosis and inhibition of MMP-9.

Neovascularization is an integral part of the infarct healing process. MI increased capillary density in the border zone as compared to sham group. Interestingly, IL-10 treatment further increased CD31-positive capillaries at 28 days post-MI. Reports suggest that suppression of IP-10 synthesis during the healing phase may allow formation of the wound neovessels, a critical process for infarct healing.37 In the present study, although IL-10 suppressed IP-10 mRNA expression, whether enhanced neovascularization in IL-10–treated infarcts was mediated through suppression of IP-10 is not clear. TNF-α has been shown to have different effects on proliferation and differentiation (tube formation) of human umbilical vein endothelial cells.38 TNF-α showed both proangiogenic (at low TNF-α concentration for a short period) and antiangiogenic (at high TNF-α concentration for a long period) effects. Because a high level of TNF-α was expressed in the myocardium, antiangiogenic effects as mentioned above might be a possibility in the present study. MMP plays an important role in angiogenesis. IL-10 inhibited MMP-9 levels and increased neovascularization in the myocardium at 28 days post-MI. This process is not clear, but reports have suggested that MMP inhibition reduces left ventricular remodeling but did not inhibit angiogenesis after MI.39,40 Although MMPs can directly stimulate angiogenesis, MMPs can also generate inhibitors of angiogenesis such as angiostatin.41 But no such attempt to look at angiostatin was made in the present study.

Several studies have shown involvement of inflammatory mediators in progressive myocytes loss caused by necrosis and/or apoptosis, suggesting that these cytokines are involved in the progression of cardiac remodeling.28,42 In the present study, the number of apoptotic myocytes increased after 28 days post-MI as compared to the sham group, but there was no significant difference between the 2 MI groups. However, it is interesting to note that there was increased number of TUNEL-positive cells lining the capillaries after MI, which was significantly lower in mice treated with IL-10 (data not shown). The role of IL-10 in apoptosis cannot be overlooked for the important reason that MI group showed increased p38 MAPK activation and reduced STAT3 phosphorylation, the 2 important pathways that play a role in apoptosis.13,14 p38 MAPK mediates both death signaling and functional depression in the heart.13 The p38 MAPK pathway indeed mediates myocardial apoptosis and functional depression by caspase-1, caspase-3, and caspase-11 activation and TNF, IL-1β, IL-6 production after myocardial ischemia.13 In a rat model of myocardial injury, inhibition of p38 MAPK lead to cardioprotective effects with improvement of cardiac function, reduction of inflammatory cell infiltration, and cardiomyocyte apoptosis.1,11,12 Therefore, in the present study, IL-10–mediated attenuation of LV dysfunction and remodeling might be attributable, in part, to suppression of p38 phosphorylation.

STAT3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury.14 Cardiomyocyte-restricted knockout of
STAT3 resulted in higher sensitivity to inflammation, cardiac fibrosis, and heart failure. STAT3-deficient mice treated with lipopolysaccharide demonstrated significantly more cardiac TNF production, apoptosis, and fibrosis. Cardiomyocyte-restricted STAT3 transgenic mice showed reduced myocardial capillary density and increased interstitial fibrosis followed by dilated cardiomyopathy with impaired cardiac function and premature death. STAT3-deficient mice showed enhanced susceptibility to MI with increased cardiac apoptosis, increased infarct sizes, and reduced cardiac function and survival. In the present study, STAT3 activation in the IL-10–treated group was associated with increased expression of VEGF-A and capillary density. Furthermore, inhibition of STAT3 with cucurbitic I in HUVECs resulted in reduced IL-10–mediated VEGF-A expression. Taken together, our study suggests that IL-10–mediated increased angiogenesis in LV after MI might be through STAT3 activation.

Although not specifically used for cardiovascular disease, the powerful immunomodulatory properties of IL-10 and the promising results from IL-10 therapy on the course of several inflammatory diseases in experimental models induced the interest on clinical application of IL-10. To our knowledge, human recombinant IL-10 has been tested in healthy volunteers and patients with Crohn’s disease, rheumatoid arthritis, psoriasis, hepatitis C infection, and HIV infection and for the inhibition of therapy-associated cytokine release in organ transplantation and Jarisch–Herxheimer reaction. These and other studies established the safety of IL-10 treatment; however, the results from clinical trials met with varied success. The varied response may represent the limiting effect of mode of therapeutic IL-10 delivery (systemic versus local) and the large size of protein (recombinant IL-10). A better understanding of IL-10 mode of action and identification of downstream targets (such as HuR and or p38 MAPK in our studies) that may mediate the effects of this cytokine may in future be suitable for small molecule based pharmacological interventions. Also, understanding the role of IL-10 in transplanted progenitor cells in the heart could enhance cell-based therapeutic approaches in cardiac interventions.

In conclusion, the data presented here suggest that IL-10 reduces severity of proinflammatory responses and contributes to improved LV function and remodeling with effects on MMP-9 activation, fibrosis, and angiogenesis after MI, possibly via the activation of STAT3 and suppression of p38 MAPKs.

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Disclosures
None.

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