Deficiency of TIMP-1 exacerbates LV remodeling after myocardial infarction in mice

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MATRIX METALLOPROTEINASES (MMPs) are a fundamental proteolytic system responsible for extracellular matrix protein degradation within the myocardium. Under ambient conditions, the myocardial MMPs largely reside in their latent form, whereas after a pathological stimulus the pool of latent MMPs becomes activated, as has been demonstrated for human, rat, and porcine myocardium (3, 6, 33, 34). Prevention of the breakdown of the myocardial extracellular matrix with pharmacological broad-spectrum MMP inhibitors in animal models of cardiomyopathy and myocardial infarction (MI) has demonstrated effects on the left ventricular (LV) remodeling (4, 20, 27, 32). An important endogenous regulator of overall MMP activity are the tissue inhibitors of MMPs (TIMPs) (18, 19). Normally, the TIMPs are in delicate balance with the MMPs and matrix is digested in a highly regulated fashion. A loss of TIMP-mediated inhibitory control has also been reported to occur in several cardiac pathologies (1, 25). For example, in end-stage cardiomyopathic disease in humans, increased MMP activity is paralleled by decreased TIMP-1, -3, and -4 expression (18). In a preliminary report, it was demonstrated that in TIMP-1-null mice, LV chamber enlargement occurred as a function of age, suggesting that constitutive expression of TIMP-1 is necessary for the maintenance of LV myocardial geometry (29). This study tested the hypothesis that TIMP-1 deficiency would modify the LV chamber and myocardial matrix remodeling after MI compared with age-matched wild-type (WT) mice, despite identical MI size.

METHODS

Experimental animals. The mice used in these studies were adult inbred 129 Sv mice from which a stable line of TIMP-1-deficient (TIMP-1−/−) mice was created through the use of a replacement vector carrying a stop codon-containing oligonucleotide and the neo resistance cassette with the 5′ end of the TIMP-1 coding frame (23, 29). The original breeding pairs used to develop the mice for this study were a kind gift from Dr. Paul J. Soloway (Roswell Park Cancer Institute, Buffalo, NY). Tail clips and a PCR protocol were used to confirm the genotype. All studies were done on age-matched mice (2 mo of age), and the groups were balanced with respect to myocardial remodeling; pressure-volume loops
to gender. MI was induced surgically by chronic ligation of the coronary artery in TIMP-1−/− mice (n = 29) and age-matched littermates (n = 33) according to recently described methods (21). Nonoperated TIMP-1−/− mice (n = 15) and littermates (n = 12) served as controls. Terminal studies, including echocardiography, LV conductance volumetry, morphometry, and histology were performed 14 days after MI, as described below. All animals were treated and cared for in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, 1996).

Echocardiographic measurements. Echocardiography was performed before MI and 2 wk after MI. Mice were sedated (40 mg/kg ketamine and 2 mg/kg xylazine) and placed in a recumbent position on a warming blanket to maintain ambient body temperature. Heart rate was determined from a surface ECG. From a transthoracic approach described previously (9, 29, 31), two-dimensional targeted echocardiographic recordings were obtained with an optimized 15-MHz transducer (15-6L Ultrasound Band; Agilent Technologies, Ab- byville, SC) integrated with a digital imaging system (Sonos 5500 Ultraharmonic Imaging System; Agilent Technologies). The two-dimensional parasternal long-axis view of the LV was recorded to precisely define the LV long axis and papillary muscles. The LV endocardial border was manually defined and LV areas at end diastole were computed by planimetry.

LV conductance volumetry. A 1.1-mm steel endotracheal tube was placed, and the anesthetized mice were mechanically ventilated (MiniVent 845; Hugo Sachs/ Harvard Apparatus, March-Huguetten, Germany). The mice were positioned on a feedback temperature-controlled operating table (Vestavia Scientific, Birmingham, AL). Under microscopic guidance (Zeiss OPMI), the right carotid artery was exposed. A precalibrated four-electrode pressure sensor catheter (1.4-Fr, SPR-839; Millar Instruments, Houston, TX) was positioned in the LV. The catheter was interfaced to a pressure-conductance unit (ARIA, MPCU-200; Millar Instruments), in which electrical excitation was performed under digital control (DAQ, PV Analysis Software; Millar Instruments). The continuous digital pressure and conductance signals were integrated with an ECG signal (PowerLab; AD Instruments) and displayed in real time with a dual-display heads-up control (DAQ, PV Analysis Software; Millar Instruments). The saline bolus volume was continuously acquired. The saline bolus caused a significant LV volume shift without a marked change in LV systolic pressure (Fig. 1A). The isochronal LV systolic and diastolic volumes were plotted along with the line of identity (Fig. 1B). From the intersection of these two lines, a mean value of 26 μl was computed for Vp. This factor was used in the software algorithms (PVAN; Millar Instruments) to compute absolute LV volumes. This saline calibration was repeated on a weekly basis in both WT and TIMP-1−/− mice to ensure that the computed Vp remained within 10% of predicted values.

Steady-state LV pressures and conductance volumetry were determined with the ventilator suspended, and the signals were digitized for a minimum of 12 consecutive cardiac cycles. From these signals, the following were determined: LV peak systolic pressure, LV end-diastolic pressure, LV peak positive developed pressure (dP/dt max), LV end-diastolic volume, LV stroke volume, and LV ejection fraction (10, 11, 36). LV radii of the LV wall thickness of the septal region and the posterior free wall was computed with a spherical frame of reference. For this purpose, LV wall thickness measurements were determined by planimetry from the perfusion fixed sections and LV peak systolic pressure and volumes were determined by the conductance catheter.

After these steady-state measurements, LV preload alterations were performed through infusion of 1 μg/kg of phenylephrine to compute the LV end-systolic pressure-volume (P-V) relationship (ESPVR). Briefly, a minimum of five isochronal LV end-systolic pressure values was plotted on the y-axis and the end-systolic volume was plotted on the x-axis. The slope of this relationship was determined with linear regression. This relationship can be used as an index of LV contractile function in mice (10, 11, 36). Because LV ESPVR can be influenced by intrinsic differences in LV geometry, this relationship was normalized to ambient LV end-diastolic volume.

Immunohistochemistry and morphometry. At completion of the hemodynamic studies, 0.5 ml of 0.1 mM cadmium chloride were injected into the LV to arrest the hearts in diastole. After perfusion with PBS, hearts were excised, weighed, routinely processed, and imbedded in paraffin. Infarct size, LV diameter, thickness of the infarcted wall, and collagen deposition were studied 2 wk after surgery in the TIMP-1−/− and WT groups with a computerized morphometry system (Quantimet 570; Leica, Cambridge, UK) on AZAN- or Sirius red-stained sections (21). Myocyte cross-sectional area was determined on hematoxylin and eosin-

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**Fig. 1. Determination of the parallel conductance volume coefficient (Vp)** to compute absolute left ventricular (LV) volumes (8, 10, 11, 15, 36). Vp was calculated by injection of an isotonic saline bolus, which caused a significant LV shift without marked changes in LV systolic pressure (A). The isochronal LV systolic and diastolic volumes were plotted along with the line identity (B). From the intersection of these two lines, a mean value of 26 μl was computed for Vp. es, End systolic; ed, end diastolic.
stained slides with computer-assisted methods described previously (33). Immunohistochemistry was performed to quantify macrophages (moma-2 monoclonal antibody on cryostat sections; Ref. 17), endothelial cells (anti-thrombomodulin polyclonal antibody; kindly provided by Dr. Peter Carmeliet, Center for Transgene Technology and Gene Therapy, Leuven, Belgium), T cells (anti CD-3; DAKO), smooth muscle cells, and myofibroblasts (mouse anti-human a-smooth muscle actin; DAKO). Cell numbers were counted per 0.1-mm² infarcted area by light microscopy at ×40 magnification with a grid.

Statistics. Data are expressed as means ± SE. Indexes of function and geometry were compared in controls and at 2 wk after MI in TIMP-1⁻/⁻ and WT groups with ANOVA, with post hoc mean separation performed by Bonferroni bounds. Means between groups were compared with the use of the Mann-Whitney U-test. A value of P < 0.05 was considered statistically significant.

RESULTS

Overall survival after MI surgery was comparable between WT and TIMP-1⁻/⁻ mice; however, it may be notable that those TIMP-1⁻/⁻ mice that did die died earlier after MI surgery than the WT mice. Of the 33 WT and 29 TIMP-1⁻/⁻ mice that underwent MI surgery, 3 WT (10%) and 8 TIMP-1⁻/⁻ (27%) mice did not recover from their anesthesia after surgery. In the WT group, eight additional animals died between days 2 and 10 because of LV rupture (n = 3) or acute heart failure (n = 5), as judged by postmortem findings (large infarct, cardiac dilatation, pleural effusion, and severe lung congestion). In the TIMP-1⁻/⁻ group, no animals were lost after the first day after surgery. The MI sizes determined by quantitative morphometric planimetry were identical between the WT and TIMP-1⁻/⁻ mice. [MI size: 42 ± 3% (WT) vs. 42 ± 3% (TIMP-1⁻/⁻) of the LV circumference].

LV geometry and function. Long-axis LV echocardiography indicated that LV dilatation was significantly increased in the TIMP-1⁻/⁻ group in response to MI, and representative echocardiograms are shown in Fig. 2A. The absolute values for LV end-diastolic area (LVEDA) were obtained from echocardiograms from WT and TIMP-1⁻/⁻ mice before and 2 wk after MI. In WT mice, LVEDA was 140 ± 6 × 10⁻³ cm² and increased to 176 ± 8 × 10⁻³ cm² in response to MI (P < 0.05). In the TIMP-1⁻/⁻ mice, LVEDA was 163 ± 4 × 10⁻³ cm² and significantly increased to 201 ± 5 × 10⁻³ cm² after MI. Both LVEDA values were significantly elevated in TIMP-1⁻/⁻ groups compared with the corresponding WT groups (both P < 0.05; n = 11–15 per group).

LV P-V loops were obtained under steady-state conditions for WT and TIMP-1⁻/⁻ mice. At 2 wk after MI, definable changes in the P-V loops were observed in both WT and TIMP-1⁻/⁻ mice (Fig. 2B). As can be seen from Fig. 2, a parallel rightward shift of the P-V loop occurred after MI in both WT and TIMP-1⁻/⁻ mice; however, in the TIMP-1⁻/⁻ mice, this rightward shift was greater. LV end-diastolic volume increased after MI in both groups of mice but was higher in the TIMP-1⁻/⁻ mice compared with the WT mice (Fig. 2C).

In addition, LV end-diastolic pressure increased significantly in the TIMP-1⁻/⁻ mice at 2 wk after MI compared with age-matched controls or with WT post-MI values (Fig. 2, B and D). Other hemodynamic parameters are summarized in Table 1. Under control conditions, ambient mean arterial pressure and LV peak pressure were slightly but significantly higher in the TIMP-1⁻/⁻ mice compared with the WT mice. Although LV architectural remodeling occurred in both WT and TIMP-1⁻/⁻ mice after MI, LV ejection fraction was...
unchanged from respective control values. LV dP/dt max appeared higher in the control TIMP-1+/mice but decreased after MI. The time constant of isovolumic relaxation (τ) was significantly prolonged in the TIMP-1−/− mice after MI and was not significantly affected in the WT group.

LV radial systolic stress at the posterior free wall was similar between control WT and TIMP-1−/− mice. LV radial systolic stress at the septum was similar to posterior free wall values in control WT and TIMP-1−/− mice (207 ± 48 and 234 ± 28 g/cm²). After MI, LV radial systolic stress at the posterior free wall increased by over sixfold in both WT and TIMP-1−/− mice and remained unchanged at the septum from control values in both WT and TIMP-1−/− groups (Fig. 3). Thus, the increased LV dilatation in the TIMP-1−/− mice after MI was not translated into increased radial wall stress because of the parallel increase in wall thickness (i.e., mass).

The LV ESPVR was computed as an index of LV contractile function. There was no difference in ESPVR between WT and TIMP-1−/− control mice (0.032 ± 0.009 and 0.030 ± 0.007 dyn·cm·mg·mmHg−1, respectively; P = 0.83). After MI, the ESPVR significantly fell to a similar degree in both WT and TIMP-1−/− mice compared with reference controls (0.016 ± 0.004 and 0.014 ± 0.005 dyn·cm·mg·mmHg−1, respectively; P < 0.05). Thus, despite a preservation of steady-state LV ejection fraction, LV contractility was significantly reduced in both WT and TIMP-1−/− groups after MI.

**LV myocardial morphometry.** Ventricular mass was significantly larger in the TIMP-1−/− mice compared with the WT mice in both control and MI animals. The absolute increase in ventricular mass in response to MI was higher in the TIMP-1+/− group, indicating that the hypertrophic response was more pronounced in TIMP-1+/− than in WT mice (Fig. 4A). Cross-sectional areas of LV myocytes were measured in a circumferential orientation in both WT and TIMP-1−/− mice and also revealed an increased hypertrophic response after MI in the TIMP-1−/− group (Fig. 4B).

The fibrillar collagen contents and cellular composition of the infarcts are summarized in Table 2. No differences were found in the densities of the total cell numbers, the number of macrophages, myofibroblast-like cells, T cells, and endothelial cells per square millimeter of infarct between the WT and TIMP-1−/− mice. With Sirius red staining, the percentage of the myocardial section occupied by fibrillar collagen was computed. Extensive collagen deposition was found in the center of the infarcts, with no differences observed between TIMP-1−/− and WT mice. However, in the septum of TIMP-1−/− control animals, we found significantly lower collagen percentages than in WT controls, indicating that there was a difference in structural composition of the hearts at the starting point of the experiment. These reduced collagen levels, together with the observed increase in infarct length in the TIMP-1−/− infarcts (Table 2), indicate that the remodeling of the infarct region is altered in the TIMP-1−/− mice.

**DISCUSSION**

Although alterations in MMP and TIMPs have been identified in end-stage human heart failure, it has remained unclear whether interruption in the balance between MMPs and TIMPs within the myocardium can directly influence the LV architectural remodeling process after specific pathological stimuli such as MI. The

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**Table 1. Hemodynamics from conductance catheter**

<table>
<thead>
<tr>
<th>Heart rate, beats/min</th>
<th>Wild Type</th>
<th>MI</th>
<th>Control</th>
<th>TIMP KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao mean, mmHg</td>
<td>85 ± 5</td>
<td>89 ± 5</td>
<td>102 ± 5</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>Stroke volume, µL/beat</td>
<td>21 ± 2</td>
<td>34 ± 2*</td>
<td>26 ± 3</td>
<td>46 ± 4*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>61 ± 1</td>
<td>64 ± 1*</td>
<td>60 ± 1</td>
<td>64 ± 1*</td>
</tr>
<tr>
<td>LV peak pressure, mmHg</td>
<td>110 ± 4</td>
<td>111 ± 4</td>
<td>127 ± 5</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>8,900 ± 600</td>
<td>8,300 ± 800</td>
<td>10,600 ± 700</td>
<td>7,600 ± 500*</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>8,900 ± 600</td>
<td>-8,600 ± 400*</td>
<td>-10,500 ± 500*</td>
<td>-8,390 ± 500*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>7.2 ± 0.4</td>
<td>8.5 ± 0.4</td>
<td>7.7 ± 0.4</td>
<td>9.5 ± 0.5*</td>
</tr>
<tr>
<td>PRSW, dyn·cm·mmHg−1</td>
<td>58 ± 25</td>
<td>37 ± 9*</td>
<td>78 ± 17</td>
<td>40 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. TIMP, tissue inhibitor of matrix metalloproteinase; KO, knockout; MI, myocardial infarction; LV, left ventricular; dP/dt max, peak positive developed pressure; dP/dt min, peak negative developed pressure; τ, time constant of isovolumic relaxation; PRSW, preload recruitable stroke work. *P < 0.05 vs. age-matched control (no MI); †P < 0.05 vs. wild-type control; ‡P < 0.05 vs. wild-type MI.
Fig. 4. Hypertrophic parameters. Total ventricular mass was corrected for tibial length to take into account any intrinsic differences in mouse size. Both total ventricular mass (A) and the cross-sectional areas of LV myocytes (B) were increased in the TIMP-1−/− group compared with the age-matched WT controls at 2 wk after MI. *P < 0.05 vs. age-matched controls; †P < 0.05 vs. WT-MI mice; &P < 0.05 vs. WT controls.

The present study demonstrated that a loss of function of TIMP-1 in mice caused significant alterations in LV geometry and function with respect to the post-MI remodeling process. Specifically, the present study demonstrated that in TIMP-1−/− mice, a greater degree of LV dilation occurred, which was accompanied by a greater hypertrophic response, increased LV filling pressures, and abnormalities in active myocardial relaxation.

The majority of past studies that have examined the LV architectural remodeling process in mice after MI have used transthoracic echocardiography (13, 30). The present study employed this technique and demonstrated that significant LV dilation occurred in mice after induction of an MI, and the degree of LV dilation that occurred in the WT mice is consistent with past reports (21, 27). In age-matched TIMP-1−/− mice, LV echocardiographic dimensions were larger than in WT mice, which was expected from past observations from this laboratory (29). However, it is difficult to assess absolute LV volumes from echocardiographic measurements, particularly after MI. Accordingly, the present study used conductance volumetry to more carefully assess indexes of LV geometry and function in mice after MI. As expected, LV volumes were significantly greater in mice after MI in both WT and TIMP-1−/− groups but were disproportionally higher in TIMP-1−/− mice after MI. Despite the significant LV chamber remodeling that occurred in both WT and TIMP-1−/− mice, LV ejection fraction was preserved compared with referenced control values. The present studies were performed at 14 days after MI, and therefore it remains to be established whether a longer time interval after MI would have resulted in the development of decreased LV ejection fraction. However, results from the present study did demonstrate abnormalities in underlying LV contractile function, which would suggest that the development to overt LV failure would be likely with longer followup periods. Specifically, a significant fall in LV ESPVR occurred in both WT and TIMP-1−/− mice after MI. Whether the significant LV chamber remodeling that occurred in the TIMP-1−/− mice after MI coupled with the diminished contractile function would accelerate the progression to LV pump failure remains to be established and warrants further study.

In the present study, a loss of myocardial matrix integrity may have directly affected myocardial contractile performance. Specifically, the maintenance of myocardial fiber alignment and geometry within the

Table 2. LV myocardial morphometrics

<table>
<thead>
<tr>
<th>Masses</th>
<th>Wild Type</th>
<th>TIMP KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>21 ± 1</td>
<td>23 ± 13</td>
</tr>
<tr>
<td>Tibial length, mm</td>
<td>16.5 ± 0.1</td>
<td>16.4 ± 0.1</td>
</tr>
<tr>
<td>Lung weight/tibial length, mg/mm</td>
<td>10.1 ± 1</td>
<td>12.6 ± 0.7*</td>
</tr>
</tbody>
</table>

LV morphometry

<table>
<thead>
<tr>
<th>Infarct size, %</th>
<th>42 ± 3</th>
<th>42 ± 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diameter, mm</td>
<td>3.6 ± 0.6</td>
<td>4.3 ± 0.4*</td>
</tr>
<tr>
<td>Thickness infarcted wall, mm</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Infarct length, mm</td>
<td>5.8 ± 1.3</td>
<td>6.8 ± 1.4*</td>
</tr>
<tr>
<td>Collagen deposition</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Noninfarcted septum, %</td>
<td>0.5 ± 0.3*</td>
<td>0.6 ± 0.3*</td>
</tr>
<tr>
<td>Infarcted region, %</td>
<td>62 ± 12</td>
<td>60 ± 13</td>
</tr>
</tbody>
</table>

Cellular composition of infarcts

| Cell density, cells/mm² | 9,100 ± 1,800 | 8,900 ± 1,700 |
| Macrophones, %          | 7.3 ± 2.4     | 6.8 ± 1.7     |
| Myofibroblasts, %       | 1.7 ± 1.4     | 1.6 ± 2.3     |
| T cells, %              | 0.2 ± 0.1     | 0.3 ± 0.1     |
| Endothelial cells, %    | 5.8 ± 1.8     | 4.4 ± 1.4     |
| Sample size             | 12             | 15             |

Values are means ± SE. *P < 0.05 vs. age-matched control (no MI); †P < 0.05 vs. wild-type control; ‡P < 0.05 vs. wild type.
LV free wall is determined, at least in part, by the myocyte-integrin-matrix interface (28). The architecture of the myocardial collagen matrix with respect to cardiac muscle alignment was described in early electron microscopy studies (2, 26). In addition, realignment of cardiac muscle fibers was reported previously to occur in rodent models of MI (24). Thus defects in the myocyte-matrix relationship within the post-MI myocardium could significantly influence the transduction of myocyte shortening into muscle fiber shortening.

There are several possible mechanisms for the relatively preserved LV ejection fractions in the current mouse model. First, in this 129 Sv mouse strain, the development of overt LV pump failure may take longer to develop than those reported previously. Second, LV ejection fraction is highly dependent on loading conditions. The present study demonstrated that an index of LV systolic function was significantly reduced in both WT and TIMP-1−/− MI mice. Thus a defect in LV function was demonstrated in the present study. Third, the present study cannot discount whether the MI or catheterization procedure superimposed on the MI caused mitral regurgitation. This low impedance outlet would cause a pseudonormalization of LV ejection fraction. This possibility is supported by the fact that the LV ESPVR was reduced in the MI mice. Thus future studies that directly determine whether this murine model of MI causes significant papillary muscle injury leading to mitral regurgitation is warranted. This is particularly important because MR in and of itself can cause significant LV chamber remodeling and influence local MMP/TIMP myocardial levels (22).

Myocardial hypertrophy occurred in the TIMP-1−/− mice after MI, as evidenced by greater ventricular mass and myocyte cross-sectional area. Because a greater degree of LV dilation occurred in the TIMP-1−/− mice, this hypertrophic response was likely due to increased stress placed on the viable myocardium. However, recent results have demonstrated that TIMPs may have multiple biological effects with respect to cell growth and viability (12). Thus the greater degree of myocardial hypertrophy in the TIMP-1−/− mice was likely multifactorial. The increased LV volumes and hypertrophy that occurred in the TIMP-1−/− mice after MI likely gave rise to specific changes in LV diastolic function. Specifically, LV end-diastolic pressure was increased and myocardial active relaxation (τ) was prolonged in TIMP-1−/− mice after MI. The mechanism for the prolongation of isovolumic relaxation time in TIMP-1−/− mice is likely to be multifactorial. Isovolumic relaxation can be influenced by LV loading conditions, different degrees of hypertrophy, and abnormalities in calcium handling/reuptake. It is likely that all of these were present in the post-MI mice. Moreover, the degree of LV hypertrophy was greater in the TIMP-1−/− mice after MI, which would likely be manifested as a prolongation in myocardial active relaxation. However, whether and to what degree other processes, such as calcium handling, are affected to a more severe degree in TIMP-1−/− mice remain to be examined.

The fibrillar collagen matrix forms the structural backbone of the myocardium (2). This network provides strength and stiffness to the myocardium and also contributes to the maintenance of myocyte alignment and geometry (35). Disruption of the structural collagens has been implicated in the pathophysiology of dilated cardiomyopathy. Recently, Kim and colleagues (16) demonstrated that direct disruption of the extracellular matrix in the heart by chronic myocardial overexpression of MMP-1 resulted in marked deterioration of systolic and diastolic function at 12 mo of age. TIMP-1 deficiency, which theoretically also results in increased MMP activity, has comparable cardiac effects. In the present study, LV sections were prepared for morphological assessment of the MI wound healing response as well as characterization of cellular and extracellular remodeling. Thus LV myocardial samples were not available for careful biochemical assessment of MMP levels and activation states. Four TIMP subtypes have been identified to date, which may be differentially regulated in the progression of the heart failure process (18). TIMPs may play several biological roles in tissue remodeling, including growth regulation (12). In light of the results of the present study, future research regarding the specific effects of TIMP-1 deletion on relative myocardial MMP levels and the expression of alternative TIMP proteins after the induction of MI is warranted.

In a previous study (29) we demonstrated that TIMP-1−/− mice, in the absence of an underlying myocardial disease, had reduced content of myocardial fibrillar collagen at 4 mo of age. In the present study, we confirmed these results and additionally showed that these TIMP-1−/− hearts, with an inadequate fibrillar collagen matrix, exhibited more extensive LV dilatation in response to MI. Thus loss of fibrillar collagen at the starting point of our experiments (i.e., at the moment of MI induction) may have been an important contributory factor for the alterations in LV geometry in the TIMP-1−/− mice after MI.

The egress of inflammatory cells from the vasculature, as well as the migration of inflammatory cells through the infarcted tissue, is dependent on a number of orchestrated steps, including the activation of proteases. These protease systems are highly redundant, but reduction in the activity of MMP-9 or the serine proteinase plasmin has been shown to modify the inflammatory response after MI in mice (5, 7, 14). The present study demonstrates that TIMP-1 deficiency does not have an apparent effect on the inflammatory response of the heart. This may be due to the fact that at 2 wk after MI the initial inflammatory response is largely completed or because other TIMP molecules may have compensated for the lack of TIMP-1. However, on the basis of the observations that fibrillar collagen was reduced and that the length of the MI was larger in the TIMP-1−/− mice, we conclude that structural remodeling of the MI region was altered in the absence of TIMP-1.

In the control state, ambient mean arterial pressure was higher in the TIMP-1−/− mice. The TIMP-1−/−
murine construct was a global gene deletion and therefore potentially may have influenced vascular structure and function. Specifically, alterations in extracellular matrix structure may influence vascular resistive properties. In the present study, LV stroke volume was also slightly higher in the control TIMP-1--/-- mice. Although remaining speculative, these factors likely contributed to the differences in arterial pressure observed in the control TIMP-1--/-- mice. Future studies that directly examine vasomotor tone and vascular structure in these TIMP-1--/-- mice will be necessary to more carefully address this issue.

In conclusion, this study emphasizes the importance of local endogenous control of MMPs by TIMP-1 for the remodeling process of the heart after MI, not only with respect to extracellular matrix structure but also with respect to myocyte growth and myocardial function. Furthermore, the results from the present study emphasize the therapeutic potential for modifying post-MI architectural remodeling through local regulation of myocardial MMP activity.

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