Effect of a Cleavage-Resistant Collagen Mutation on Left Ventricular Remodeling

Merry L. Lindsey,* Jun Yoshioka,* Catherine MacGillivray, Suphichaya Muangman, Joseph Gannon, Anjali Verghese, Masanori Aikawa, Peter Libby, Stephen M. Krane, Richard T. Lee

Abstract—Matrix metalloproteinase–mediated degradation of type I collagen may play a role in cardiac remodeling after strain or injury. To explore this hypothesis, we used mice homozygous (r/r) for a targeted mutation in Colla1; these mice synthesize collagen I that resists collagenase cleavage at Gly975-Leu976. A total of 64 r/r and 84 littermate wild-type mice (WT) underwent experimental pressure overload by transverse aortic constriction (TAC) or myocardial infarction (MI). Echocardiographic, hemodynamic, and histological parameters were evaluated up to 12 weeks after TAC or 21 days after MI. At 4 weeks after TAC, collagen levels, wall thickness, and echocardiographic parameters were similar in the 2 groups. At 12 weeks after TAC, r/r mice had smaller LV dimensions (ESD: 2.7±0.2 mm WT versus 1.7±0.2 mm r/r, P<0.013; EDD: 3.8±0.2 mm WT versus 3.1±0.1 mm r/r, P<0.013); better fractional shortening (30±2% WT versus 46±4% r/r; P<0.013); and lower LV/body weight ratios (7.3±0.6 WT and 5.1±0.5 r/r; P<0.013). Surprisingly, these differences were not accompanied by differences in collagen accumulation, myocyte cross-sectional areas, wall thickness, or microvessel densities. Furthermore, no differences in LV remodeling assessed by echocardiography, fibrosis, or hemodynamic parameters were found between r/r and WT mice after MI. Thus, a mutation that encodes a collagenase cleavage-resistant collagen I does not affect early LV remodeling after TAC or MI, suggesting that collagen cleavage at this site is not the mechanism by which metalloproteinases mediate LV remodeling. Collagen cleavage could, however, have a role in preservation of cardiac function in late remodeling by mechanisms independent of collagen accumulation. We were not able to detect collagen cleavage fragments, and could not, therefore, rule out the possibility of collagen cleavage at additional sites. (Circ Res. 2003;93:238-245.)

Key Words: matrix metalloproteinases ■ collagen ■ left ventricular remodeling ■ myocardial infarction

Left ventricular (LV) remodeling is an important cause of congestive heart failure despite the use of therapeutic agents that inhibit angiotensin II or β-adrenergic signaling, highlighting the need for additional understanding of remodeling events.1 Matrix metalloproteinases (MMPs) comprise a family of enzymes that degrade extracellular matrix and may mediate cardiac remodeling. The expression of certain MMPs increases in nonischemic dilated cardiomyopathy2 and after myocardial infarction (MI), in the presence or absence of reperfusion.3,4 Drugs that act as “nonselective” MMP inhibitors prevent ventricular dilation in mice, rats, pigs, and rabbits.5-8 In addition, deletion or overexpression of MMPs in mice regulates cardiac remodeling and hypertrophy.9-11

The potential roles of MMPs in cardiac remodeling have not yet been fully elucidated. Collagen is the most abundant protein in the body, and collagen I represents about 90% of total collagen.12 MMP effects on collagen turnover, therefore, may explain effects on remodeling. One hypothesis is that MMPs degrade collagen fibrils and allow cardiomyocytes to “slip” and then establish new matrix attachments.13 This hypothesis could explain the ventricular dilation that occurs in mice with transgenic overexpression of human MMP-1,11 a collagenase, the orthologue of which is expressed only at low levels in mice.14 Alternatively, MMP inhibition could affect the metabolism of a noncollagen molecule that is important in cardiac remodeling. A recent study of selective MMP inhibition supports this hypothesis; administration of an MMP-1 sparing MMP inhibitor prevented LV remodeling after MI in rabbits, a species that utilizes MMP-1 for collagenolysis.8

To provide insight into these mechanisms, we examined the effects of type I collagen cleavage on cardiac remodeling by using mice with a mutation targeted to the region of the Colla1 gene that encodes amino acid substitutions around a site (Gly975-Leu976) in the helical domain of the collagen I α1(I) chains where collagenase cleavage commonly occurs.15
The r/r mice produce collagen I molecules in which neither the α1(I) nor the α2(I) chains are cleaved by collagenases. Although the Col1α1 mice have a strikingly diminished response to parathyroid hormone-stimulated osteoclastic bone resorption, ventricular remodeling has not been studied in these mice. We demonstrate in this study that, although this mutation does not affect the transition to hypertrophy after pressure overload, the later transition to dilatation is reduced in the r/r mice. Surprisingly, the r/r mice exhibit no differences in early remodeling events after myocardial infarction, and the mutation does not affect total collagen accumulation in remodeling myocardium.

Materials and Methods

Animal Care

All animal procedures were conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996) and approved by the Animal Research Committee of Harvard Medical School.

Mice

The Col1α1 mice were derived from the Jl/129 strain and backcrossed >6 generations into the C57BL/6 background. We used the heterozygous progeny of heterozygous breeding pairs of Col1α1tmLαs mice to generate additional backcrosses. Littermate offspring were identified by genotyping using a PCR-based method described previously.

Transaortic Constriction (TAC) and Myocardial Infarction (MI)

TAC was produced as described previously. Echocardiographic acquisition and analysis were performed by an echocardiographer blinded to treatment group. Light anesthesia with spontaneous respiration was achieved with intraperitoneal pentobarbital (10 mg/kg). All images were taken at a heart rate greater than 400 bpm to minimize effects of anesthesia, using a Sonos 4500 (Philips) and a 15-MHz transducer. Three consecutive cardiac cycles were averaged. LV mass was calculated by the M-mode (cubic) method. The calculated and actual weights consecutive cardiac cycles were averaged. LV mass was calculated at a heart rate greater than 400 bpm to minimize effects of anesthesia, /H11005/P (taken at autopsy) had excellent correlation (/H11006/P = 0.4% (n /H9262/5) to 1.2% (n /H9262/6) to 15.1% (n /H9262/17; /H11005/P = 0.102). The r/r mice produced collagen I molecules in which neither Col1α1 nor the Col1α1 chains are cleaved by collagenases. The 9A4 antibody was a generous gift of Peter G. Mitchell (Pfizer Central Research). Staining, scanning, and quantitation were all performed in a blinded manner using a minimum of 5 sections for each animal.

Statistical Analysis

Data are presented as mean+/−SEM and were analyzed for differences between WT and r/r mice at discrete time points. Normally distributed data were analyzed by the unpaired t test; data not normally distributed were analyzed by the Mann-Whitney test. To account for the 4 between-group comparisons, /H11005/P <0.013 was considered significant.

Results

Col1α1r/r Mice Have Similar Survival Rates and Normal Early Cardiac Remodeling After TAC

Of 69 r/r and 69 WT mice that underwent the TAC procedure, 10 r/r and 12 WT mice died perioperatively (within 12 hours after surgery). In addition, 4 r/r and 1 WT mice died after anesthesia administration for echocardiographic studies. Of the 55 r/r and 56 WT mice that remained, 15 r/r and 7 WT mice died during the protocol. These rates were not statistically different by Fisher’s exact test (P = 0.102).

To determine whether cleavage resistance regulates collagen accumulation after TAC, both interstitial (Figures 1A and 1C) and perivascular areas (Figures 1B and 1D) were compared. Picrosirius red–stained sections were quantified for the percent area stained positive for collagen (interstitial fibrosis) or for the amount of collagen surrounding a vessel, divided by the lumen area (perivascular fibrosis). At 4 weeks, WT had an expected increase in interstitial collagen from an unoperated value of 1.2±0.4% (n = 5) to 15.1±2.9% (n = 16), and r/r mice had a surprisingly similar increase from 2.3±0.8% (n = 6) to 15.1±2.6% (n = 17; /H11005/P = NS). There were also no differences between groups at 8 or 12 weeks after TAC. WT had collagen to lumen area ratios that increased from 0.3±0.1 (n = 5) in unoperated controls to 1.8±0.4 (n = 16) at 4 weeks, whereas r/r mice had an increased ratio from 0.7±0.2 (n = 6) to 1.7±0.3 (n = 18; /H11005/P = NS). There were no differences in perivascular fibrosis between the groups at 4, 8, or 12 weeks. These data demonstrate that WT and r/r mice have similar fibrotic responses to TAC and suggest that type I collagenase cleavage at the Gly975-Leu976 site is not necessary during the development of myocardial or perivascular fibrosis.

To determine if cleavage resistance of collagen affects myocyte hypertrophy, myocyte cross-sectional areas were quantified. The hypothesis that myocyte expansion requires collagen I cleavage rests on the concept that collagen I degradation provides space for the myocyte to “slip.” Myocyte cross-sectional areas increased from unoperated values of 123±40 μm² (n = 5) to 4 weeks after TAC values of 320±25 μm² (n = 16) for WT and 142±22 μm² (n = 6) to 337±19 μm² (n = 18) for r/r (P = NS). In addition to increased myofibrillar width, changes in myocyte nuclei furnish another measure of hypertrophy. Both an increase in nuclei numbers and the presence of enlarged hyperchromatic myocyte nuclei occur in response to pressure overload. Nuclei area in unoperated mice was 4.6±1.0% for WT (n = 5) and...
4.2±0.4% for r/r mice (n=6). At 4 weeks, the hematoxylin-positive area had increased to 9.5±8% for WT (n=16) and 9.0±9% for r/r mice (n=18; P=NS). The increased nuclei areas did not differ statistically between the 2 groups at any time point. In summary, both groups had similar responses in myocyte growth.

Previous in vitro studies have suggested that new vessel growth requires collagen I cleavage. To test the hypothesis that r/r have reduced vessel density, the area positive for biotinylated Griffonia (Bandeiraea) Simplicifolia Lectin-I (GSL, Vector Laboratories; 50 μg/mL), an endothelial cell marker, was quantified. Vessel area in unoperated LVs was 12.6±1.1% for WT (n=4) and 11.6±2.1% for r/r mice (n=6; P=NS). At 4 weeks after TAC, both groups had an increase of approximately 3%. WT had vessel areas of 15.1±0.9% (n=16), whereas r/r had vessel areas of 14.3±1.0% (n=18). There were no differences in endothelial density at any time point, demonstrating that collagenase resistance at this site did not impair new vessel growth after TAC.

**Collagenase-Resistant Mice Have Reduced LV Dilation at 12 Weeks After TAC**

To test the hypothesis that collagenase resistance contributes to decreased LV function after TAC, echocardiographic and hemodynamic parameters were measured in WT and r/r mice at 4, 8, and 12 weeks after TAC (Figures 2 and 3). There were no differences between WT and r/r mice in heart rate, body weight, or wall thickness (Table 1). End-diastolic dimensions (EDD), end-systolic dimensions (ESD), and fractional shortening (FS, %) were increased in WT at 4 weeks after TAC, and r/r mice had similar increases in dimensions. In contrast, r/r had reduced dilation compared with WT at 12 weeks (EDD: 3.8±0.2 mm for WT [n=18] versus 3.1±0.1 mm for r/r [n=13], P<0.013; ESD: 2.7±0.2 mm for WT [n=18] versus 1.7±0.2 mm for r/r [n=13], P<0.013; and LV mass to body weight ratio: 7.3±0.6 for WT [n=16] versus 5.1±0.5 for r/r [n=13], P<0.013). Both groups had similar increases in LV mass, anterior wall thickness, posterior wall thickness, and relative wall thickness (RWT=2·...
PWT/EDD). These increases in LV mass and wall thickness occurred in the first 4 weeks. Based on echocardiography, r/r maintained their ventricular dimensions through the 12-week time point. In contrast, WT had further increases in LV mass, in the absence of further increases in relative wall thickness. None of the parameters evaluated, including wall thickness, ventricular dimensions, or size, changed in unoperated mice from either group evaluated at 8, 12, or 16 weeks of age, either between groups or ages. This finding, coupled with increases in LV dimensions without increases in myocyte cross-sectional areas, indicates that WT ventricles begin to dilate. There were no differences between the 2 groups in end-diastolic pressure or LV peak developed pressure (dP/dt), suggesting that compensatory mechanisms operate in WT to maintain hemodynamics.

Collagenase Resistance Does Not Affect Infarct Size, Fibrosis, or the Hypertrophic Response to MI

To test the hypothesis that collagen I cleavage at the Gly975-Leu976 site contributes to decreased LV function after MI, echocardiographic parameters were measured in WT and r/r mice at 1, 7, and 21 days after MI, and hemodynamic measurements were taken at 21 days. Both groups experienced increased dilatation (Table 2). EDD increased from 3.6±0.1 to 3.9±0.1 mm in the WT (n=20) and from 3.3±0.1 to 3.4±0.2 mm in the r/r mice. EDD did not differ significantly between groups or ages. In contrast, fractional shortening (FS) decreased from 60±2% to 52±3% in the WT (n=20) and from 62±3% to 52±3% in the r/r mice. Fractional shortening did not differ significantly between groups or ages. These findings suggest that WT mice have progressive increases in heart weight to body weight ratios; r/r mice maintain their 4-week post-TAC values. LV mass (top) and LV mass to body weight ratio (bottom) were calculated before (n=44 WT and 44 r/r), 4 weeks (n=13 WT and 26 r/r), 8 weeks (n=17 WT and 17 r/r), and 12 weeks (n=16 WT and 13 r/r) after TAC. Data are shown as mean±SEM. *P<0.013, WT vs r/r mice.
to 3.7±0.1 mm in the r/r mice (n=16; P=NS) at 21 days. ESD increased from 2.7±0.2 to 3.1±0.2 mm in the WT (n=20) and from 2.5±0.1 to 2.9±0.2 mm in the r/r mice (n=16; P=NS) at 21 days. The LV mass to body weight ratios increased from 4.4±0.3 to 6.3±0.4 (n=20) and from 5.2±0.2 to 6.2±0.5 (n=16; P=NS) for the WT and r/r mice, respectively, at 21 days. End-diastolic pressure (EDP) was 2±1 mm Hg for both groups at 21 days (n=15 for each group; P=NS). We have noted that mice of this age do not increase EDP significantly after MI, unless the MI is massive. The relatively flat diastolic filling curve in the mouse has been reviewed by Kass.28 The τ relaxation constant was 7.8±0.8 ms for the WT and 8.8±0.9 ms for the r/r mice (P=NS; n=15 for each group). Together, these data demonstrate a similar impairment in LV function after MI in the presence or absence of collagenase-cleavable collagen I.

**TABLE 1. Echocardiographic and Hemodynamic Parameters for TAC Mice**

<table>
<thead>
<tr>
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<th>Before</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
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<tr>
<td>WT</td>
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<tr>
<td>t/r</td>
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<td>44–45</td>
<td>13–18</td>
<td>26–31</td>
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<tr>
<td>t/r</td>
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<td>44–46</td>
<td>13–18</td>
<td>26–31</td>
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<tr>
<td>Heart rate, bpm</td>
<td>611±11</td>
<td>590±12</td>
<td>615±19</td>
<td>580±18</td>
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<tr>
<td>Body weight, g</td>
<td>22±1</td>
<td>22±1</td>
<td>26±2</td>
<td>25±1</td>
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<td>Anterior wall thickness, mm</td>
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<td>0.74±0.02</td>
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<td>Posterior wall thickness, mm</td>
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<td>Relative wall thickness</td>
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<td>0.4±0.02</td>
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<tr>
<td>WT</td>
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<td>t/r</td>
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WT indicates wild type; t/r, collagenase-resistant collagen mutant; and bpm, beats per minute.

**TABLE 2. Echocardiographic and Hemodynamic Parameters for MI Mice**

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<th>1 Day</th>
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<tr>
<td>Infarct size, %</td>
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<tr>
<td>Echocardiography</td>
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<tr>
<td>WT</td>
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<tr>
<td>t/r</td>
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<tr>
<td>Heart rate, bpm</td>
<td>611±17</td>
<td>598±26</td>
<td>623±19</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24±1</td>
<td>21±1</td>
<td>25±1</td>
</tr>
<tr>
<td>Anterior wall thickness, mm</td>
<td>0.78±0.05</td>
<td>0.80±0.05</td>
<td>0.81±0.03</td>
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<tr>
<td>Posterior wall thickness, mm</td>
<td>0.87±0.03</td>
<td>0.94±0.04</td>
<td>0.99±0.04</td>
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<td>End-diastolic dimensions, mm</td>
<td>3.6±0.1</td>
<td>3.3±0.1</td>
<td>4.0±0.1</td>
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<tr>
<td>End-systolic dimensions, mm</td>
<td>2.7±0.2</td>
<td>2.5±0.1</td>
<td>3.2±0.2</td>
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<td>Fractional shortening, %</td>
<td>25±2</td>
<td>26±2</td>
<td>20±2</td>
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<td>LV mass, g</td>
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<td>0.11±0.01</td>
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<td>LV mass/BW ratio</td>
<td>4.4±0.3</td>
<td>5.2±0.2</td>
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<td>Hemodynamics, n</td>
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<td>Heart rate, bpm</td>
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<tr>
<td>End-systolic pressure, mm Hg</td>
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<td>End-diastolic pressure, mm Hg</td>
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<td>dP/dt maximum, mm Hg/s</td>
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<td>τ, ms</td>
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WT indicates wild type; t/r, collagenase-resistant collagen mutant; and bpm, beats per minute.
Discussion

In this study, WT and r/r mice had similar early changes in LV geometry in response to 2 different stimuli (TAC and MI). None of the variables monitored (fibrosis, hypertrophy, or LV function) differed significantly between the 2 groups at early time points, suggesting that cleavage of collagen I or LV function differed significantly between the 2 groups at MI). None of the variables monitored (fibrosis, hypertrophy, or LV geometry in response to 2 different stimuli (TAC and MI). In this study, WT and r/r mice had similar early changes in LV geometry in response to 2 different stimuli (TAC and MI). None of the variables measured in these experiments, and collagen III, which is not cleavage-resistant, may play an important role in the late phase.

Early MMP inhibitor clinical trials had severe but reversible musculoskeletal pain and inflammation side effects, suggesting that selective MMP inhibition that does not block MMP-1 may be better tolerated. More recent evidence suggests, however, that inhibition of non-MMP metalloproteinases (such as sheddase activity of the ADAMs family proteases) is responsible for the musculoskeletal side effects. These clinical trials emphasize the need for more information on disease and stage-specific MMP profiles to direct effective therapeutic use of MMP inhibition. The roles of MMPs in cleavage or activation of nonmatrix substrates is also poorly understood. MMPs can

These results indicate that initial MMP effects on LV remodeling do not depend on collagenolysis of collagen I at the Gly975-Leu976 site. These findings contrast with those in the dermis and uterus of r/r mice, where excessive collagen deposition does occur, perhaps emphasizing the effects of tissue specificity. Dermal fibroblasts cultured in the presence of ascorbate require collagen cleavage for MMP-2 activation independent of the presence of MT-1 MMP or serpin, aspartyl, or cysteine enzymatic activity, suggesting a role for collagen degradation in MMP activation. We did not observe an increase in MMP-8 or MMP-13 levels in the LV of either WT or r/r mice 7 days after TAC.

The r/r mice did not develop dilation after more prolonged exposure to pressure overload. At 12 weeks after TAC, WT mice had increased LV weights, without increased wall thickness, and decreased function, reflecting a transition from the compensated nondilated hypertrophic state to dilation. Although collagen cleavage may not affect LV hypertrophy, it may contribute to LV dilation. Thus, the surrounding collagen fibrils may limit an increase in myocyte length during dilation, whereas an increase in wall thickness does not require MMP-regulated cleavage. Several other laboratories have monitored MMP levels during the progression to heart failure. Using a rat model of infrarenal abdominal aortocaval fistula, Chancey et al. observed LV dilation 8 weeks after volume overload that was blunted with the MMP inhibitor PD166793. Dahl salt-sensitive hypertensive rats still in concentric hypertrophy had no change in MMP-2, TIMP-2, or TIMP-4. The transition to heart failure, in contrast, was accompanied by increased MMP-2, TIMP-2, and TIMP-4. Although they did not evaluate collagenase levels, the trend is similar in that increased MMP/TIMP levels are associated with increased dilation. Li et al. observed increased LV dilation with a concomitant increase in MMP-13 and decrease in TIMP-4 in the spontaneously hypertensive heart failure rat model. Although LV dilation and MMP-13 levels was attenuated by treatment with either PD166793 or an ACE inhibitor (quinapril), collagen mRNA levels and hypertrophy decreased only in the quinapril-treated group, suggesting distinct pathways for the development of hypertrophy and dilation. Specific subtypes of collagen were not measured in these experiments, and collagen III, which is not cleavage-resistant, may play an important role in the late phase.

Discussion

In this study, WT and r/r mice had similar early changes in LV geometry in response to 2 different stimuli (TAC and MI). None of the variables monitored (fibrosis, hypertrophy, or LV function) differed significantly between the 2 groups at early time points, suggesting that cleavage of collagen I by collagenases at the Gly975-Leu976 site does not contribute to early remodeling. Eleftheriades et al. demonstrated that fibrillar collagen accumulation in a young rat abdominal aortic banding model was the result of a reduced rate of intracellular collagen degradation rather than increased collagen synthesis. In their model, collagen I mRNA levels did not increase until 16 weeks after banding, suggesting that regulation at the degradation step occurs first. Thus, it is possible that reduced collagen cleavage at sites other than Gly975-Leu976 contributes to collagen accumulation in the myocardium. Our laboratory has previously demonstrated that a selective MMP-1–sparing MMP inhibitor improved LV remodeling and function at 4 weeks after MI. Other collagenases (MMP-8, MMP-13, and MMP-14) cleave collagen at the Gly975-Leu976 site; in addition, MMPs can cleave collagen at other sites. We were not able to detect collagen cleavage fragments, and could not, therefore, rule out the possibility of collagen cleavage at these additional sites.

These results indicate that initial MMP effects on LV remodeling do not depend on collagenolysis of collagen I at the Gly975-Leu976 site. These findings contrast with those in the dermis and uterus of r/r mice, where excessive collagen deposition does occur, perhaps emphasizing the effects of tissue specificity. Dermal fibroblasts cultured in the presence of ascorbate require collagen cleavage for MMP-2 activation independent of the presence of MT-1 MMP or serinyl, aspartyl, or cysteinyl enzymatic activity, suggesting a role for collagen degradation in MMP activation. We did not observe an increase in MMP-8 or MMP-13 levels in the LV of either WT or r/r mice 7 days after TAC.

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Early MMP inhibitor clinical trials had severe but reversible musculoskeletal pain and inflammation side effects, suggesting that selective MMP inhibition that does not block MMP-1 may be better tolerated. More recent evidence suggests, however, that inhibition of non-MMP metalloproteinases (such as sheddase activity of the ADAMs family proteases) is responsible for the musculoskeletal side effects. These clinical trials emphasize the need for more information on disease and stage-specific MMP profiles to direct effective therapeutic use of MMP inhibition. The roles of MMPs in cleavage or activation of nonmatrix substrates is also poorly understood. MMPs can
regulate a broad range of cellular functions such as proliferation, differentiation, malignant transformation, and death. Potential nonmatrix MMP substrates that have a role in the early response to MI include cytokines, growth factors and their receptors, adhesion molecules, mediators of apoptosis, and angiogenic factors. The glycoproteins fibronectin and osteopontin are both increased after MI.

The late phase differences suggest that collagen I cleavage plays additional roles other than maintaining matrix organization. For instance, collagen fragments generated by MMP-1 in bone may have active osteoclasts. The potential release or exposure of binding sites present but not normally exposed in matrix proteins (matricryptic sites) is not known for collagen I, although other matrix molecules (e.g., laminin-5 and collagen IV) have matricryptic sites that promote cell migration. Other MMPs (particularly MMP-9) may play a predominant role in early remodeling events, whereas MMP-1 may have a more prominent role during late remodeling. The mutation engineered in Coll1a1 in the r/r mice encodes changes in several amino acids around the collagenase cleavage site. Dzamba et al found that the r/r collagen binds with less avidity to fibronectin-Sepharose than WT collagen. Fibronectin normally binds collagen I or gelatin in the region encoded by the targeted mutation. Thus, although the r/r mutation was designed to encode collagenase resistance, it also alters collagen binding to fibronectin.

In summary, collagen cleavage resistance does not affect early LV remodeling after TAC or MI, suggesting that early LV remodeling occurs through a collagen cleavage–independent mechanism. In contrast, collagen I breakdown may contribute to depressed cardiac function in late remodeling by a collagen accumulation–independent mechanism, possibly through control of myocyte length via a direct effect on the collagen surrounding the myocyte. Alternatively, secondary effects on expression or activity of other matrix or protease components may affect the remodeling process. These studies emphasize the need to look beyond the assigned MMP substrates to examine MMP physiological functions.

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In an article by Lindsey et al (Circ Res, 2003;93:238–245), the charts in Figure 1C and 1D were inadvertently transposed in the final version of the manuscript. Also, the collagenase cleavage site in the α1(I) chains of type I collagen was incorrectly referred to throughout the manuscript as Gly975-Leu976. The correct position of the cleavage site is Gly775-Ile776. The authors apologize for these errors.