Targeted Overexpression of Noncleavable and Secreted Forms of Tumor Necrosis Factor Provokes Disparate Cardiac Phenotypes

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Background—Recent studies suggest that posttranslation processing or “shedding” (ie, secretion) of tumor necrosis factor (TNF) by tumor necrosis factor-α converting enzyme (TACE) may contribute to the left ventricular (LV) remodeling that occurs in the failing human heart.

Methods and Results—To address the functional significance of TNF shedding, we generated lines of transgenic mice with targeted overexpression of secreted wild-type (MHCsTNF2) TNF and overexpression of a mutated noncleavable transmembrane form of TNF (MHCmTNF). Both lines of mice had overlapping levels of myocardial TNF protein; however, the phenotypes of the MHCsTNF2 and MHCmTNF mice were strikingly disparate. Whereas the MHCmTNF mice developed a concentric LV hypertrophy phenotype, the MHCsTNF2 mice developed a dilated LV phenotype. The fibrillar collagen weave in MHCmTNF mice with concentric hypertrophy was characterized by thick collagen fibrils and increased collagen content, whereas the fibrillar collagen weave in the MHCsTNF2 mice with LV dilation was characterized by a diminished collagen content. Inhibition of matrix metalloproteinases with a broad-based matrix metalloproteinase inhibitor prevented LV dilation in the MHCsTNF2 mice.

Conclusions—These findings suggest that posttranslational processing of TNF, as opposed to TNF expression per se, is responsible for the adverse cardiac remodeling that occurs after sustained TNF overexpression. (Circulation. 2004;109:262-268.)

Key Words: hypertrophy • remodeling • metalloproteinases

Recent studies from this and other laboratories have suggested that the expression of tumor necrosis factor (TNF) in the heart confers cytoprotective responses (reviewed in Mann1). These salutary effects of TNF have been difficult to reconcile with the known untoward effects of sustained TNF expression, which include left ventricular (LV) dysfunction, extracellular matrix degradation, and LV remodeling, any or all of which may contribute directly to the development of cardiac decompensation (reviewed in Mann2). Relevant to the present discussion is the observation that TNF is initially synthesized as a 26-kDa type II transmembrane protein that is subsequently cleaved (ie, secreted) by a membrane-bound enzyme termed TNF-α converting enzyme (TACE).3 The resultant 17-kDa TNF monomer that is “shed” from the cell-surface membrane subsequently assembles as a biologically active homotrimer that initiates cell signaling.3 Noting that the membrane-bound and secreted forms of TNF have distinctly different biological actions4 and that TACE is upregulated in the heart in pathophysiological contexts wherein LV dilation and dysfunction occur,5 we questioned whether posttranslational processing of TNF (ie, secretion) might contribute to the adverse cardiac remodeling that is observed after sustained TNF expression. In the present report, we compared a line of transgenic mice with targeted cardiac overexpression of a mutated form of TNF that lacks the TACE recognition site, and hence cannot be secreted, with a second line of mice with targeted cardiac overexpression of wild-type secreted TNF.

Methods

Generation and Characterization of MHCmTNF and MHCsTNF, Transgenic Lines

Lines of transgenic mice with cardiac restricted overexpression of secretable TNF (referred to as MHCsTNF2 mice) and cardiac

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262
restricted overexpression of a mutated form of TNF that lacked the TACE recognition site (referred to as MHCmTNF mice) that is responsible for TNF shedding (secretion) were generated with the α-myosin heavy chain (MHC) promoter (a gift from Jeff Robbins, University of Cincinnati, Cincinnati, Ohio) to target TNF expression to the cardiac myocyte, as described previously. The TNF transgene that encoded wild-type secretable TNF contained an intact TACE recognition site, whereas the transgene coding for the noncleavable transmembrane form of TNF lacked the TACE recognition site (see Data Supplement). The generation and preliminary characterization of the MHCmTNF has been reported previously. Both the MHCmTNF and MHCsTNF mice were generated on an FVB background to facilitate comparison between lines of transgenic mice.

Characterization of Cardiac Phenotype in MHCmTNF and MHCsTNF Mice

Cardiac Phenotype and Morphology

Perfusion fixation and histological analysis of the hearts were performed as described (see Data Supplement). Myocyte length was measured on fixed isolated cardiac myocytes according to the method of Gerdes et al.

Cardiac Structure and Hemodynamics

We used 2D-targeted M-mode echocardiography and simultaneous hemodynamic catheterization to characterize LV structure and hemodynamics, respectively, in male wild-type, MHCmTNF, and MHCsTNF mice. LV end-systolic and end-diastolic pressure were assessed with a 1.4F microtipped Millar catheter. LV end-systolic and end-diastolic wall stress were calculated with hemodynamic data and the assessments of end-systolic and end-diastolic LV wall thickness that were obtained from M-mode echocardiography.

Myocardial Fibrillar Collagen

Scanning electron microscopy and picrosirius red staining were performed to examine myocardial fibrillar collagen, as described previously.

Matrix Metalloproteinase Activity and Abundance

Matrix metalloproteinase (MMP) zymographic activity was measured in myocardial protein extracts (16 μg) with gelatin zymography. Purified gelatinase (MMP9, 0.125 mg/mL) and MMP2 (0.125 mg/mL) were used as standards. To measure MMP2 abundance (ie, the sum of latent and activated MMP2 species), the cardiac protein extracts were treated with β-aminophenylmercuric acetate (APMA), an organomercurial compound that activates MMPs. MMP2 abundance was then measured with an ELISA that measures levels of total activated MMP2 species (#RPN 2631, Amersham).

Expression of Tissue Inhibitor of Metalloproteinases

Type 1

Tissue inhibitor of metalloproteinases type 1 (TIMP-1) levels were measured in myocardial extracts by ELISA (#RPN 2611, Amersham), as described previously.

MMP Inhibition

To determine the effects of MMP inhibition on LV structure in MHCsTNF, mice, we treated MHCsTNF mice with an MMP inhibitor (MMPI, PD 166793 (30 mg·kg⁻¹·d⁻¹) in chow), from 3 to 6 weeks of age (n=13/group). Six-week-old wild-type mice (n=13) and 6-week-old MHCmTNF, mice (n=11) fed normal chow were used as the appropriate controls. In preliminary dosing studies in this strain of mice, this dosing strategy resulted in steady state plasma levels of 33±2 μg/mL. This plasma level of PD 166793 exceeds the concentration necessary to inhibit all of the classes of MMPs. Importantly, PD 166973 has no effect on other metalloproteinases such as TACE. After the 3-week treatment period, the mice were anesthetized, and a calibrated 4-electrode pressure sensor catheter (1.4F, Millar Instruments) was positioned in the LV. The catheter was interfaced to a Millar volumetric-conductance unit to compute absolute LV volumes in vivo, as described previously.

Statistical Analysis

All values are expressed as mean±SEM. Two-way ANOVA was used to detect differences between MHCmTNF, MHCsTNF, and wild-type mice for all parameters at the ages studied. Where appropriate, post hoc testing was performed with a Tukey test to detect differences between groups. Significant differences were said to exist at P<0.05.

Results

Characterization of MHCmTNF and MHCsTNF Mice

We obtained 5 heterozygous founder lines that harbored cardiac restricted overexpression of the wild-type secretable TNF transgene: s49 (6 copies), s112 (12 copies), s118 (32 copies), s121 (32 copies), and s122 (32 copies). Each of these lines developed a dilated cardiac phenotype (data not shown). The s49 line was selected for further characterization, insofar as the myocardial TNF protein levels overlapped those observed in a previously characterized MHCmTNF line of mice. The level of myocardial TNF protein in MHCmTNF mice was not significantly different (P=0.9) from that detected in MHCsTNF mice at 6 and 24 weeks of age (Figure 1A). As expected, myocardial TNF protein levels in both MHCmTNF and MHCsTNF mice were significantly greater (P<0.001) than those observed in age-matched wild-type mice. Western blot analysis cardiac extracts from MHCsTNF mice revealed a prominent 26-kDa band, corresponding to the transmembrane form of wild-type TNF, and a fainter band corresponding to the 17-kDa secreted form of TNF (Figure 1B). In contrast, cardiac extracts from MHCmTNF mice revealed a prominent 24-kDa band, corresponding to the noncleavable transmembrane form of TNF protein that lacked the TACE recognition site. We did not detect a 17-kDa band in the cardiac extracts from the MHCmTNF lines of mice. Plasma levels of circulating TNF were significantly (P<0.001) increased in MHCsTNF mice at 6 and 24 weeks of age (P<0.001) compared with MHCmTNF and wild-type mice at comparable ages (Figure 1C), whereas plasma TNF levels in MHCmTNF and wild-type mice were barely detectable and were not significantly different from each other (P=0.33).

Phenotypic Characterization of MHCmTNF and MHCsTNF Mice (6 Weeks)

Figure 2 shows that at 6 weeks of age, the MHCmTNF mice developed a concentric cardiac hypertrophic phenotype (Figures 2E and 2H), whereas the MHCsTNF mice developed a dilated cardiac phenotype (Figures 2F and 2I). Histological examination of hearts from the MHCmTNF (Figure 2K) and MHCsTNF (Figure 2L) mice showed a normal linear arrangement of myofibrils compared with wild-type mice (Figure 2J). There was no obvious myofibrillar disarray nor prominent interstitial infiltrating cells in any of the sections that were examined from the MHCmTNF and MHCsTNF mice.

Longitudinal Characterization of Cardiac Phenotype in MHCmTNF and MHCsTNF Mice (6 to 24 Weeks)

Figure 3 summarizes the longitudinal assessment of cardiac structure in the wild-type, MHCmTNF, and MHCsTNF mice.
mice. The heart-weight-to-body-weight ratios were significantly greater ($P=0.002$) in the MHCmTNF and MHCsTNF$_2$ mice than in the wild-type mice at 6 and 24 weeks of age (Figure 3A). This increase in heart-weight-to-body-weight ratio in the MHCmTNF mice and the MHCsTNF$_2$ mice was due, at least in part, to a significant ($P<0.006$) increase in LV mass-to-body-weight ratio in the MHCmTNF and MHCsTNF$_2$ mice compared with the wild-type control mice (Figure 3B). However, the pattern of cardiac hypertrophy was different in the MHCmTNF and MHCsTNF$_2$ mice. Consistent with the phenotypic appearance of the hearts presented in Figure 2, MHCmTNF mice developed a hypertrophic phenotype that was characterized by a significant ($P<0.001$) increase in LV wall thickness (ie, concentric hypertrophy) compared with wild-type mice (Figure 3C), whereas MHCsTNF$_2$ mice developed a cardiac phenotype characterized by a significant ($P<0.001$) increase in LV volume compared with wild-type mice (Figure 3D). Importantly, the concentric hypertrophic phenotype of the MHCmTNF mice persisted from 6 to 24 weeks, as shown by the preserved increase in LV wall thickness, with no increase in LV

Figure 1. Characterization of MHCmTNF and MHCsTNF$_2$ mice (6 to 24 weeks). A, Myocardial TNF protein (pg/mg of total protein) in FVB wild-type, MHCmTNF, and MHCsTNF$_2$ mice (n=5 to 6 per group). B, Western blot analysis of myocardial protein extracts from wild-type, MHCsTNF$_2$, and MHCmTNF mice. Recombinant human TNF (rhTNF) was used as standard for 17-kDa secreted TNF protein. C, Serum TNF protein (pg/ml) in wild-type, MHCmTNF, and MHCsTNF$_2$ mice (n=5 to 6 per group).

Figure 2. Cardiac phenotype of wild-type, MHCmTNF, and MHCsTNF$_2$ mice (6 weeks). A through C, Representative photographs of whole hearts; D through F, corresponding coronal sections of hearts from wild-type, MHCmTNF, and MHCsTNF$_2$ mice, respectively. G through I, Representative hematoxylin-and-eosin-stained cross sections at level of papillary muscles from wild-type, MHCmTNF, and MHCsTNF$_2$ mice, respectively. J through L, Representative hematoxylin-and-eosin-stained myocardial sections ($\times400$) from wild-type, MHCmTNF, and MHCsTNF$_2$ mice, respectively.
end-diastolic volume (Figures 3C and 3D). Similarly, the dilated cardiac phenotype of the MHCsTNF 2 mice was maintained, as demonstrated by the persistent ($P < 0.001$) increase in LV volume at 6 to 24 weeks in the MHCsTNF 2 mice compared with the wild-type and the MHCmTNF mice (Figure 3D).

**Longitudinal Characterization of Myocardial Structure in MHCmTNF and MHCsTNF 2 Mice (6 and 24 Weeks)**

**Cardiac Myocyte Hypertrophy**

The cross-sectional area of myocytes from the hearts of MHCmTNF mice was significantly ($P < 0.05$) greater in MHCmTNF mice at 6 and 24 weeks than in wild-type and MHCsTNF 2 mice, whereas myocyte cross-sectional area was not significantly different ($P > 0.05$) between MHCsTNF 2 mice and wild-type control mice (Table 1). In contrast, myocyte cell length was significantly increased ($P < 0.05$) in MHCsTNF 2 mice at 6 and 24 weeks compared with both wild-type and MHCmTNF mice, whereas myocyte cell length was not significantly different ($P = 0.67$) between MHCmTNF and wild-type mice (Table 1).

**Myocardial Fibrillar Collagen**

Figures 4B and 4E show that there was a loss of myocardial fibrillar collagen in the MHCsTNF 2 mice at 6 and 24 weeks.

![Figure 3. Longitudinal characterization of LV structure in wild-type, MHCmTNF, and MHCsTNF 2 mice group data (6 to 24 weeks). A, Ratio of heart weight to body weight (n=6 animals per group per time point). B, LV mass-to-body-weight ratio (n=6 hearts per group per time point). *$P<0.001$ vs wild-type. LV wall thickness (C) and end-diastolic volume (D; n=6 to 9 hearts per group per time point). *$P<0.001$ vs wild-type and MHCmTNF mice.](http://circ.ahajournals.org/)

### TABLE 1. Cardiac Myocyte Morphometry in Wild-Type, MHCmTNF, and MHCsTNF 2 Mice

<table>
<thead>
<tr>
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<th>Myocyte Cross-Sectional Area, $\mu m^2$</th>
<th>Myocyte Length, $\mu m$</th>
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<tbody>
<tr>
<td></td>
<td>6 Weeks</td>
<td>24 Weeks</td>
</tr>
<tr>
<td>Wild type*</td>
<td>212.8±2.7</td>
<td>224.0±1.4†</td>
</tr>
<tr>
<td>MHCmTNF*</td>
<td>271.4±2.1‡</td>
<td>249.9±2.2‡</td>
</tr>
<tr>
<td>MHCsTNF 2</td>
<td>213.6±3.4</td>
<td>225.0±3.1</td>
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Myocyte cross-sectional area was measured in fixed tissue (n=6 to 11 hearts per group). Myocyte length was measured in intact myocytes obtained by KOH digestion of formalin-fixed hearts (n=35 to 45 myocytes obtained from 4 hearts per group).

*Data on myocyte cross-sectional area in MHCmTNF and wild-type mice have been reported previously” and are shown here for the sake of comparison to MHCsTNF 2 mice.

†$P<0.05$ vs wild-type mice.

‡$P<0.05$ vs comparable line of mice at 6 weeks of age.
of age compared with age-matched wild-type controls. In contrast, myocardial sections taken from the MHCmTNF mice revealed a dense fibrillar collagen weave and a thickening of the fibrillar struts between myocytes (Figures 4C and 4F). To further characterize the time-dependent changes in fibrillar collagen content, we performed picrosirius red staining in the MHCsTNF, MHCmTNF, and wild-type mice (n=5 to 6 hearts per time point). There was a significant (P<0.002) 25% to 35% decrease in myocardial fibrillar collagen content in MHCsTNF mice compared with MHCmTNF and wild-type control mice (Figure 4G), which coincided with the qualitative changes in fibrillar collagen weave observed in the MHCsTNF mice (Figures 4B and 4E), whereas there was a significant (P<0.001) 35% to 40% increase in collagen content in the MHCmTNF mice at 6 and 24 weeks of age (compared with age-matched control mice), consistent with the qualitative assessment of fibrillar collagen in the MHCmTNF mice (Figures 4B and 4E).

Mechanism for the Disparate Cardiac Phenotypes in MHCmTNF and MHCsTNF2 Mice

**Hemodynamic Assessment of MHCmTNF and MHCsTNF2 Mice**

Peak LV systolic pressure was not significantly different between the MHCmTNF, MHCsTNF2, and wild-type mice (Table 2). Moreover, end-systolic wall stress was significantly less (P<0.05) in the MHCmTNF mice (25.99±1.5 g/cm²) than in either the MHCsTNF2 mice (31.10±1.2 g/cm²) or the wild-type mice (37.75±1.0 g/cm²). LV end-diastolic pressure was significantly (P<0.05) elevated in MHCsTNF2 compared with MHCmTNF and wild-type mice.  

**MMP Activity**

As shown in the representative gelatin zymograms in Figure 5A, we observed lytic bands at ~65 kDa (arrow) in all 3 lines of mice, suggestive of MMP2 activity. However, relative to wild-type mice and MHCmTNF mice, MMP activity was significantly greater (P<0.001 and P<0.02, respectively) in MHCsTNF2 mice at 6 weeks of age, coinciding with the decrease in fibrillar collagen content observed in these mice at this time point. There was, however, no significant (P=0.11) overall difference in MMP activity between wild-type and MHCmTNF mice. In addition, there was a striking decrease in MMP activity in MHCsTNF2 mice at 24 weeks of age (P<0.007 for 6 weeks versus 24 weeks), whereas MMP activity was not significantly different (P=0.32) in MHCmTNF mice from 6 to 24 weeks of age. To determine whether the changes in MMP activity observed in MHCsTNF2 mice were the result of changes in MMP2 abundance, we measured MMP2 abundance at 6 and 24 weeks of age. Figure 5C shows that there was a significant increase (P<0.001) in MMP2 abundance in both the MHCsTNF2 mice and the MHCmTNF mice compared with wild-type mice at 6 weeks of age. However, whereas MMP2 abundance decreased in MHCsTNF2 mice and was not significantly different (P>0.4) different from wild-type mice at 24 weeks of age, MMP2 abundance remained increased in MHCmTNF mice and was significantly (P<0.001) greater than in wild-type mice at 24 weeks of age. Insofar as there was no significant difference in total MMP2 abundance between MHCmTNF and MHCsTNF2 hearts at 6 weeks (P=0.94), the increase in MMP2 zymographic activity in MHCsTNF2 mice suggests there was increased activation of MMP in these mice.

**TABLE 2. Patterns of LV Wall Stress in Wild-Type, MHCmTNF, and MHCsTNF2, Mice (6 Weeks)**

<table>
<thead>
<tr>
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<th>LV Peak Systolic Pressure, mm Hg</th>
<th>LV End-Diastolic Pressure, mm Hg</th>
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<tr>
<td>Wild type</td>
<td>75.3±1.8</td>
<td>16.0±1.0</td>
</tr>
<tr>
<td>MHCmTNF</td>
<td>75.6±1.2</td>
<td>16.9±1.4</td>
</tr>
<tr>
<td>MHCsTNF2</td>
<td>78.1±1.5</td>
<td>23.4±1.3*</td>
</tr>
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</table>

*P<0.05 vs wild-type; †P<0.05 vs MHCmTNF; ‡P<0.05 vs MHCsTNF2.
TIMP Levels
We next examined changes in TIMP-1 levels as a possible mechanism for the observed differences in MMP activity in the MHCmTNF and MHCsTNF2 mice. Figure 5D shows that relative to levels in wild-type mice, TIMP-1 levels were significantly lower in MHCsTNF2 and MHCmTNF mice at 6 weeks of age ($P = 0.04$ and $P = 0.002$, respectively). However, at 24 weeks of age, there was a significant ($P = 0.90$) difference in TIMP-1 levels between MHCsTNF2, MHCmTNF, and wild-type mice.

MMP Inhibition
As shown in Table 3, treatment of MHCsTNF2 (n=13) mice with an MMPi from 3 to 6 weeks of age significantly ($P < 0.05$) attenuated the degree of LV dilation compared with untreated MHCsTNF2 mice (n=13). Furthermore, attenuation of LV dilation was not the result of a decrease in afterload, insofar as the peak LV systolic pressures were not significantly different ($P = 0.27$) in wild-type, MMPi, and MHCsTNF2 mice.

Discussion
This study, in which we compared lines of transgenic mice with targeted overexpression of a transgene coding for noncleavable transmembrane TNF (MHCmTNF) develop a concentric LV hypertrophy phenotype (Figures 2, 3A, 3B, and 3C), whereas mice with targeted overexpression of a transgene coding for secreted TNF (MHCsTNF2) develop a dilated LV hypertrophy phenotype (Figures 2, 3A, 3B, and 3D). Importantly, the disparate patterns of LV hypertrophy in MHCmTNF and MHCsTNF2 mice were not secondary to differences in myocardial TNF protein levels, which were not significantly different in the 2 lines of mice (Figure 1A). Moreover, the observed differences in cardiac phenotype did not appear to be the result of selective differences in LV afterload between the 2 models, insofar as peak LV end-systolic pressure was not significantly different in MHCmTNF and MHCsTNF2 mice (Table 2). Rather, the disparate patterns of LV cardiac hypertrophy in MHCmTNF and MHCsTNF2 mice were secondary to the manner in which the transmembrane and secreted forms of TNF selectively influenced remodeling of cardiac myocytes and the extracellular matrix. Whereas the fibrillar collagen weave in MHCmTNF mice was characterized by thick collagen fibrils (Figures 4C and 4F) and increased collagen content (Figure 4G), the fibrillar collagen weave in the mice that developed LV dilation (MHCsTNF2) was characterized by dissolution of the collagen weave (Figures 4B and 4E) and diminished collagen content (Figure 4G).

To delineate the mechanisms that were responsible for the different patterns of extracellular matrix remodeling in the MHCmTNF and MHCsTNF2 mice, we asked whether there were differences in MMP activity and TIMP levels in the 2 lines of mice, insofar as previous observations from this and other laboratories have implicated time-dependent changes in the balance between MMP activity and TIMP activity as an important determinant of LV dilation.$^{6,12}$ Consistent with our previous observations, we observed increased MMP abundance, increased MMP activity (Figures 5A and 5B), and

### Table 3. Effect of MMP Inhibition on LV Structure and Hemodynamics in MHCsTNF2 Mice

<table>
<thead>
<tr>
<th></th>
<th>LV Peak Systolic Pressure, mm Hg</th>
<th>LV End-Diastolic Volume, µL</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>88.2 ± 1.6</td>
<td>39.2 ± 2</td>
</tr>
<tr>
<td>MHCsTNF2</td>
<td>93.9 ± 2.1</td>
<td>61.4 ± 4*</td>
</tr>
<tr>
<td>MHCsTNF2 + MMPi</td>
<td>91.1 ± 2.8</td>
<td>49.4 ± 4*†</td>
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* $P < 0.05$ vs wild type (n=11); † $P < 0.05$ vs MHCsTNF2 (n=13).
decreased TIMP levels in the MHCsTNF2 mice at 6 weeks of age. Collectively, the increased levels of active MMP-2 determined by zymography coupled with the reduction in TIMP-1 levels would favor increased MMP activation within the myocardium and thereby accelerate degradation of the extracellular matrix in the MHCsTNF2 mice. Notably, the most remarkable change in MMP-2/TIMP-1 stoichiometry occurred at 6 weeks, which coincided with the significant increase in LV dilation in the MHCsTNF2 mice at this time point. Given the MMP2 abundance and that TIMP levels were not significantly different in the MHCmTNF and MHCsTNF2 mice, the increased MMP-2 activational state in the MHCsTNF2 mice was likely due to increased activation of 1 or more upstream proteases (eg, plasmin) or local MMP activational systems (eg, membrane-bound MMPs) that are known to activate MMPs. Although the potential reasons for this differential activation are not known, one plausible explanation is that transmembrane TNF is spatially constrained, whereas secretable TNF lacks spatial constraint and is able to activate upstream proteases in endothelial cells, smooth muscle cells, and fibroblasts, which in turn are capable of activating MMPs in a paracrine manner. The importance of MMP activation as a mechanism for LV dilation in the MHCsTNF2 mice was shown by studies in which an MMPi was sufficient to attenuate LV dilation in MHCsTNF2 mice (Table 3), consistent with previous reports that have shown that MMPi attenuates LV dilation in different heart failure models.13–15

Conclusions

The findings of the present study are compatible with the known pleiotropic effects of TNF and highlight the disparate roles that transmembrane and secreted TNF play in the heart. On a more basic level, these studies may also provide insight into the basic mechanisms that govern LV remodeling in the heart. That is, whereas previous studies have focused on changes in cardiac myocyte length as a primary determinant of LV remodeling, the results of the present study suggest that this view may be overly simplistic. The observation that LV dilation in MHCsTNF2 mice was dependent on permissive changes in MMP activity and TIMP levels that favored matrix degradation and was abrogated by MMP inhibition suggests that changes in myocyte length (hypertrophy) and myocardial fibrillar collagen content likely occur in tandem, as the ventricle dilates in response to changes in diastolic hemodynamic loading conditions. This in turn raises the intriguing, if not important, question of how these events are coordinated at the cellular and molecular level during cardiac remodeling. To this end, the transgenic mouse lines developed herein should provide us with useful model systems to begin to delineate the signaling events that govern the concurrent changes in myocyte growth and fibrillar collagen content during the important transition from concentric hypertrophy to dilated cardiomyopathy.

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