Selective disruption of MMP-2 gene exacerbates myocardial inflammation and dysfunction in mice with cytokine-induced cardiomyopathy

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Matsusaka, Hidenori, Masaki Ikeuchi, Shouji Matsushima, Tomomi Ide, Toru Kubota, Arthur M. Feldman, Akira Takeshita, Kenji Sunagawa, and Hiroyuki Tsutsui. Selective disruption of MMP-2 gene exacerbates myocardial inflammation and dysfunction in mice with cytokine-induced cardiomyopathy. Am J Physiol Heart Circ Physiol 289: H1858–H1864, 2005. First published June 3, 2005; doi:10.1152/ajpheart.00216.2005.—Tumor necrosis factor-α (TNF-α) plays a pathophysiological role in the development and progression of heart failure. Matrix metalloproteinase (MMP)-2 is involved in extracellular matrix remodeling. Recent evidence suggests a protective role for this protease against tissue inflammation. Although MMP-2 is upregulated in the failing heart, little is known about its pathophysiological role. We thus hypothesized that ablation of the MMP-2 gene could affect cardiac remodeling and failure in TNF-α-induced cardiomyopathy. We crossed transgenic mice with cardiac-specific overexpression of TNF-α (TG) with MMP-2 knockout (KO) mice. Four groups of male and female mice were studied: wild-type (WT) with wild MMP-2 (WT/MMP-2+/+), WT with MMP-2 KO (WT/MMP-2−/−), TNF-α TG with wild MMP-2 (TG/MMP-2+/+), and TG with MMP-2 KO (TG/MMP-2−/−). The upregulation of MMP-2 zymographic activity in TG/MMP-2+/+ mice was completely abolished in TG/MMP-2−/− mice, and other MMPs and tissue inhibitors of metalloproteinase were comparable between groups. Survival was shorter for male TG/MMP-2−/− than TG/MMP-2+/+ mice. Female TG/MMP-2−/− mice were more severely affected than TG/MMP-2+/+ mice with diminished cardiac function. Myocardial TNF-α and other proinflammatory cytokines were increased in TG/MMP-2+/+ mice, and this increase was similarly observed in TG/MMP-2−/− mice. The extent of myocardial infiltrating cells including macrophages was greater in TG/MMP-2−/− mice. Selective ablation of the MMP-2 gene reduces survival and exacerbates cardiac failure in association with the increased level of myocardial inflammation. MMP-2 may play a cardioprotective role in the pathogenesis of cytokine-induced cardiomyopathy.

metalloproteinases; heart failure; tumor necrosis factor

THE DYNAMIC SYNTHESIS and breakdown of extracellular matrix (ECM) proteins play an important role in myocardial remodeling and failure. In particular, increased expression and activation of matrix metalloproteinases (MMPs) have been implicated in heart failure (4, 8, 20, 23, 26, 27). The activity of MMPs is controlled by transcription, activation of the latent proenzymes, and inhibition of MMPs by tissue inhibitors of MMPs (TIMPs). Although transcriptional regulation is essential for MMP production, activation of latent enzymes by proteolytic cleavage is required for matrix degradation. In addition, TIMPs modulate ECM deposition through inhibition of activated MMPs. Together, activation of latent MMP and inhibition of MMPs by TIMPs contribute to myocardial remodeling and failure. Among the known MMPs, MMP-2 is ubiquitously distributed in cardiac myocytes and fibroblasts (2) and has been shown to be upregulated in heart failure (1, 8). Recent studies have shown that MMP-2 has diverse cellular function, such as attenuation of the tissue inflammatory response, independent of its action on the ECM (3, 13, 29). Therefore, the ultimate effects of MMPs may include modulation of ECM proteins, as well as modification of cellular functions, including cell migration.

Myocardial production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), plays an important role in the pathogenesis of heart failure (3, 22). Transgenic (TG) mice that overexpress TNF-α specifically in the heart developed myocardial inflammation, with premature death from heart failure in association with ECM remodeling (16, 17). Activation of MMPs, i.e., MMP-2 and MMP-9, has been demonstrated in this model (17, 18). Furthermore, expression of MMP-2 can be regulated by the inflammatory cytokines (19). Therefore, MMP-2 could influence the progression of inflammation by affecting the function of mediators such as cytokines/chemokines and could play an important role in myocardial remodeling. In the present study, we evaluated the effects of a targeted deletion of the MMP-2 gene on cardiac structural and functional alterations in this type of heart failure. To ensure selective and long-term complete inhibition of MMP-2, we crossed TNF-α TG mice with MMP-2 knockout (KO) mice (8, 12). The most effective way to evaluate the contribution of a specific MMP and obtain direct evidence for a role of MMP is through gene manipulation instead of an MMP inhibitor.

MATERIALS AND METHODS

Animal model. We used TG mice with cardiac-specific overexpression of TNF-α, which have been well characterized as a model of cytokine-induced cardiomyopathy (16). To ensure complete inhibition of MMP-2 activity, we crossed TG mice with MMP-2 KO (MMP-2−/−) mice. There were no detectable differences in cardiac size and structure between MMP-2−/− and MMP-2+/+ mice either macroscopically or microscopically (8). The original breeding pairs used to develop the mice for this study were obtained from Dr. Shigeo Chihara (Laboratory for Behavioral Genetics, RIKEN). Because

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The myocardial samples were homogenized in 1 ml of an ice-cold extraction buffer containing cadoxodicy acid (10 mmol/l), NaCl (0.15 mmol/l), ZnCl₂ (20 mmol/l), NaN₂ (1.5 mmol/l), and 0.01% Triton X-100 (pH 5.0). The homogenate was then centrifuged (4°C, 10 min, 10,000 g), and the supernatant was decanted and saved on ice. The pH levels of the samples were adjusted to 7.5 with Tris (1 mmol/l). The final protein concentration of the myocardial extracts was determined using a standard colorimetric assay. The extracted samples were aliquoted and stored at -80°C until the time of assay. The myocardial extracts were then directly loaded onto electrophoretic gels (SDS-PAGE) containing 1 mg/ml gelatin under nonreducing conditions. The myocardial extracts at a final protein content of 5 μg were loaded onto the gels using a 3:1 sample buffer (10% SDS, 4% sucrose, 0.25 mol/l Tris·Cl, and 0.1% bromophenol blue, pH 6.8). The gels were run at 15 mA/gel through the stacking phase (4%) and at 20 mA/gel for the separating phase (10%), while the running buffer temperature was maintained at 4°C. After SDS-PAGE, the gels were washed twice in 2.5% Triton X-100 for 30 min each, rinsed in water, and incubated for 24 h in a substrate buffer at 37°C (50 mmol/l Tris·HCl, 5 mmol/l CaCl₂, and 0.02% NaN₃, pH 7.5). After incubation, the gels were stained with Coomassie brilliant blue R-250. The zymograms were digitized, and the size-fractionated bands, which indicated MMP proteolytic levels, were measured by integrated optical density in a rectangular region of interest.

The mRNA levels of myocardial MMPs, including MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9, as well as TIMPs, including TIMP-1, TIMP-2, TIMP-3, and TIMP-4, were determined by multi-probe ribonuclease protection assay (RiboQuant, PharMingen). Each value was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each template set as an internal control and then calculated as a ratio to WT/MMP⁺/⁺.

Survival. Survival was analyzed in male and female WT/MMP⁺/⁺, WT/MMP⁻/⁻, TG/MMP⁻/⁻, and TG/MMP⁺/⁻ mice. During the study period, the cages were inspected daily to identify any deceased animals. All deceased mice were examined for the presence of pleural effusion (serous fluid within the chest wall cavity) in the postmortem examination. Because most of the male TG mice died earlier, we used 12-wk-old female mice for the subsequent analyses.

Echocardiographic and hemodynamic measurements. Echocardiographic studies were performed under light anesthesia with tribromoethanol-amylene hydrate (Avertin, 2.5% w/vol, 8 μg/g ip) and spontaneous respiration as described previously (24). A two-dimensional parasternal short-axis view of the LV was obtained at the level of the papillary muscles. In general, the best views were obtained with the transducer lightly applied to the midpoint of the upper left anterior chest wall. The transducer was then gently moved cephalad or caudal and angulated until desirable images were obtained. After confirmation that the imaging was on axis (on the basis of roundness of the LV cavity), two-dimensional targeted M-mode traces were recorded at a paper speed of 50 mm/s. Then a 1.4 Fr micromanometer-tipped catheter (Millar) was inserted into the right carotid artery and advanced into the LV to measure LV pressures. Our previous validation study showed that intra- and interobserver variabilities of our echocardiographic measurements for LV cavity dimensions and fractional shortening were small and that measurements made in the same animals on separate days were highly reproducible (24).

Histopathology. After in vivo echocardiographic and hemodynamic studies, the heart was excised and dissected into right ventricle and LV, including the septum. From the mid-LV transverse sections, 5-μm sections were cut and stained with hematoxylin and eosin and Masson’s trichrome for determination of myocyte cross-sectional area and collagen volume fraction. They were stained also with picrosirius red for visualization of the interstitial collagen fibers.

Myocardial infiltration was quantified in hematoxylin-and-eosin-stained sections by determination of nuclear density (nuclei/mm²) according to methods described previously (15, 21). Because it is difficult to differentiate inflammatory cells from myocytes and/or fibroblasts, all nuclei were included. In each animal, five independent high-power fields were analyzed. To further determine the number of macrophages, an immunohistochemical analysis using a specific antibody against mouse Mac-3 (BD Pharmingen) was performed.

Cytokine gene expression. To determine the myocardial gene expression of TNF-α as well as other proinflammatory cytokines, including regulated on activation, normal T cell expressed and secreted (RANTES), interleukin (IL)-6, IL-1β, transforming growth factor (TGF)-β, and monocyte chemotactic protein (MCP)-1, ribonuclease protection assay was performed with 5 μg of total RNA isolated from LV tissue samples according to methods described previously (11).

Plasma cytokine and MMP levels. Plasma levels of TNF-α, as well as other cytokines, were measured with commercially available ELISA kits for mouse TNF-α, IL-6, IL-1β, and MCP-1 (Quantikine, R & D Systems). Plasma MMP-2 was also measured with commercially available ELISA kits (Quantikine). All assays were done in duplicate. Results were analyzed spectrophotometrically at a wavelength of 450 nm with a microtiter plate reader.

Statistical analysis. Values are means ± SE. A survival analysis was performed by the Kaplan-Meier method, and between-group difference in survival was tested by the log-rank test. Between-group comparisons of the means were performed by one-way ANOVA followed by t-tests. Bonferroni’s correction was done for multiple comparisons of the means.

RESULTS

MMPs and TIMPs. Zymographic MMP-2 levels increased in TNF-α TG/MMP⁺/⁺ compared with WT/MMP⁺/⁺ mice (Fig. 1). As expected, MMP-2 activity was not detected in WT/MMP⁻/⁻ and TG/MMP⁻/⁻ mice. Importantly, the MMP-9 zymographic levels, even though they were faint in WT/MMP⁺/⁺ mice, did not increase in WT/MMP⁻/⁻ mice. They significantly increased in the TNF-α TG groups; however, no difference was seen between TG/MMP⁺/⁺ and TG/MMP⁻/⁻ mice.

Again, the MMP-2 mRNA levels significantly increased in TNF-α TG/MMP⁺/⁺ compared with WT/MMP⁺/⁺ mice (Fig. 2). This increase was completely prevented in TG/MMP⁻/⁻ mice. The MMP-9 mRNA levels also increased in the TNF-α TG groups; however, no difference was seen between TG/MMP⁺/⁺ and TG/MMP⁻/⁻ mice. These results were consistent with those observed in gelatin zymography (Fig. 1). Other MMPs, including MMP-1, MMP-3, and MMP-8, were not altered in these mice (Fig. 2). The changes in TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) were comparable between TG/MMP⁺/⁺ and TG/MMP⁻/⁻ mice.

Survival. Survival rate was shorter for male TG/MMP⁺/⁻ than TG/MMP⁻/⁻ mice (Fig. 3). Although MMP-2 gene ab-
male TG mice, because the extent of myocardial expression of 12-wk-old female mice for the subsequent analyses. Graphic and hemodynamic data of the surviving 12-wk-old (TG) mice with MMP-2 (TG/MMP KO (TG/MMP KO (TG/MMP KO) B and C; densitometric analysis of MMP-2 and MMP-9 zymographic activity (n = 3/group). Samples from WT/MMP++/+ mice were run concurrently on the same gel. Values are means ± SE. **P < 0.01 vs. WT/MMP++/+. † † P < 0.01 vs. TG/MMP++/+. female mice are shown in Table 1. Presence or absence of the MMP-2 gene did not affect baseline echocardiographic parameters in WT mice. LV end-diastolic diameter was significantly greater, and fractional shortening was significantly less in TG/MMP++/+ than in WT mice. TG/MMP+/− mice exerted more impaired LV contractile function than TG/MMP++/+ mice.

There was no significant difference in heart rate or LV end-diastolic pressure (EDP) between WT/MMP++/+ and WT/MMP+/− mice. LV EDP increased slightly, but significantly, in TG/MMP++/+ mice and further increased in TG/MMP+/− mice. Consistent with LV EDP, pleural effusion was observed only in TG/MMP+/− mice. On the basis of these results, the exacerbation of heart failure might contribute to premature death in TG/MMP+/− mice.

Histopathology. We examined the histopathology of the heart from 12-wk-old female mice. LV weight was significantly increased in the TG groups compared with the WT groups (Table 1); however, LV weight did not differ between TG/MMP++/+ and TG/MMP+/− mice. Furthermore, myocyte cross-sectional area and collagen volume fraction were increased in TNF-α TG groups (Fig. 4, A and B). However, the extent of these histopathological changes was comparable between TG/MMP++/+ and TG/MMP+/− mice. Moreover, in picrosirius red-stained sections, the structure of the interstitial collagen fibers was similar between TG/MMP++/+ and TG/ MMP+/− mice (Fig. 4C), indicating that selective disruption of the MMP-2 gene did not alter collagen content or structure in this mouse model.

The number of infiltrating interstitial cells in the myocardium was greater in TNF-α TG than in WT mice (Fig. 5). The extent of infiltration was significantly greater in TG/MMP+/− than TG/MMP++/+ mice. Macrophages infiltrated into the myocardium from TG mice, and, importantly, selective MMP-2 ablation further augmented their infiltration (Fig. 6).

Cytokine gene expression. TNF-α gene expression was significantly upregulated in the TNF-α TG myocardium (Fig. 7). In addition, overexpression of the TNF-α gene increased expression of other cytokines and chemokines, including RANTES, IL-6, IL-1β, and MCP-1, indicating that overexpression of TNF-α induced “downstream” cytokines and chemokines in this model. Importantly, upregulation of TNF-α was not altered by ablation of the MMP-2 gene. Similarly, MMP-2 gene ablation in TG mice had no significant effects on myo-
cardiac mRNA levels of other cytokines/chemokines, indicating that the decline in survival and LV function in TG/MMP−/− mice was not due to enhancement of myocardial cytokine/chemokine expression by selective disruption of the MMP-2 gene.

Plasma cytokine and MMP levels. Plasma levels of TNF-α, as well as other cytokines, were below detection in the four groups of animals (Table 2), consistent with previous studies demonstrating that the TNF-α TG transcripts were limited to the heart (16). As expected, plasma levels of MMP-2 were very low in MMP-2 KO mice. Plasma MMP-2 was comparable between WT/MMP+/+ and TG/MMP++/++ mice.

**DISCUSSION**

In the present study, we demonstrated that selective disruption of the MMP-2 gene exacerbated survival and LV function in TG mice with cardiac-specific overexpression of TNF-α. Disruption of the MMP-2 gene did not alter myocardial hypertrophy and interstitial fibrosis but exacerbated inflammatory cell infiltration. These results indicate that MMP-2 plays a protective role against myocardial inflammation and dysfunction in cytokine-induced cardiomyopathy.

Consistent with previous studies (17, 18), MMP-2 mRNA and activities were upregulated in myocardium from animals with TNF-α-induced cardiomyopathy (Figs. 1 and 2). Although the mechanisms responsible for this activation remain to be determined, cellular constituents of cardiac muscle, including fibroblasts, inflammatory cells, and myocytes, are known to be capable of expressing MMP-2 in response to specific stimuli (25). Activation of MMPs may be involved in the remodeling process of the failing heart by provoking alterations of the ECM (4, 8, 20, 23, 26, 27). Indeed, MMP-9 disruption reduced myocardial remodeling and improved LV function and survival rate after myocardial infarction (9). Similarly, MMP-2 ablation inhibited cardiac rupture and remodeling after myocardial infarction (8).

The present study investigated the long-term effects of selective MMP-2 gene disruption on development of TNF-α-induced cardiomyopathy. We employed KO mice, because selective MMP-2 inhibitors are not available and “selective” MMP-2 disruption is possible only with a KO mouse model. As expected, no MMP-2 expression was observed in myocardium from WT/MMP−/− and TG/MMP−/− mice (Figs. 1 and 2).

**Table 1. Characteristics of animal models**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT/MMP+/+ (n = 15)</th>
<th>WT/MMP−/− (n = 15)</th>
<th>TG/MMP+/+ (n = 15)</th>
<th>TG/MMP−/− (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiographic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>450 ± 11</td>
<td>446 ± 10</td>
<td>452 ± 12</td>
<td>453 ± 11</td>
</tr>
<tr>
<td>LV EDD, mm</td>
<td>3.4 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>4.3 ± 0.1†</td>
<td>4.5 ± 0.2†</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>37.4 ± 0.8</td>
<td>36.8 ± 0.7</td>
<td>26.7 ± 0.9*</td>
<td>22.9 ± 1.3†‡</td>
</tr>
<tr>
<td><strong>Hemodynamic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>471 ± 10</td>
<td>469 ± 10</td>
<td>460 ± 2.9</td>
<td>459 ± 8</td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>0.5 ± 0.7</td>
<td>0.2 ± 0.3</td>
<td>1.5 ± 0.7†</td>
<td>7.2 ± 2.5†</td>
</tr>
<tr>
<td><strong>Organ weight data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt, g</td>
<td>20.7 ± 0.5</td>
<td>19.2 ± 0.4</td>
<td>21.7 ± 0.4</td>
<td>20.7 ± 0.5</td>
</tr>
<tr>
<td>LV wt/body wt, mg/g</td>
<td>3.0 ± 0.1</td>
<td>2.9 ± 0.0</td>
<td>3.8 ± 0.1†</td>
<td>3.9 ± 0.2†</td>
</tr>
<tr>
<td>Pleural effusion, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

*Values are means ± SE. WT/MMP+/+, wild-type mice with matrix metalloproteinase-2 (MMP-2); WT/MMP−/−, wild-type mice with MMP-2 knockout; TG/MMP+/+, transgene (TG) mice with cardiac overexpression of TNF-α with MMP-2; TG/MMP−/−, TG mice with cardiac overexpression of TNF-α with MMP-2 knockout; LV, left ventricular; EDD, end-diastolic diameter; EDP, end-diastolic pressure. *P < 0.01 vs. WT/MMP+/+. †P < 0.05 vs. TG/MMP−/−.
2). We had expected that MMP-2 disruption could ameliorate cardiac ECM remodeling and dysfunction in TNF-α TG mice and prolong survival. On the contrary, selective blockade of the MMP-2 gene exacerbated LV contractile dysfunction and failure (Table 1) and even shortened survival (Fig. 3), suggesting that myocardial induction of MMP-2 may play a protective role against the development of cytokine-induced cardiomyopathy. The most striking finding of the present study was an increase in inflammatory cell recruitment into the myocardium seen in MMP-2 KO mice due to overexpression of TNF-α in the heart (Figs. 5 and 6). Even though the pathophysiological significance of cellular infiltrates in myocardial remodeling and failure remains mostly speculative in this model, the observed increase in inflammation might exacerbate myocardial contractile and structural defects, which could lead to premature death in TG/MMP−/− mice. Several potential mechanisms of MMP-2 deletion are responsible for exacerbating cellular inflammation in TNF-α TG hearts. One possible mechanism is a further increase in expression of the TNF-α gene in TG/MMP−/− mice and the resultant enhancement of inflammation. However, this possibility is less likely, because the gene expression of cytokines, TNF-α and MCP-1, was not altered by selective disruption of the MMP-2 gene (Fig. 7), perhaps in part because expression of TNF-α in TG mice is driven by α-myosin heavy chain promoter, which is supposed to be MMP-2 independent. Another possibility is that MMP-2 might alter the milieu of the ECM, which could accelerate the infiltration of cells. In the present study, no significant changes were observed in collagen deposition between TG/MMP+/+ and TG/MMP−/− mice (Fig. 4). Therefore, exacerbation of myocardial inflammation in TG/MMP−/− mice was not due to impairment of interstitial collagen formation and/or structure.

Theoretically, an increase in MMP activity would result in a decrease in the MMP substrate, collagen, whereas inhibition of MMP-2 would result in an increase in collagen. However, in agreement with previous studies (18), the present study demonstrated that an increase in MMP-2 activity was accompanied by an increase in fibrosis in TNF-α TG mice (Fig. 4). Moreover, selective disruption of the MMP-2 gene did not alter these changes in interstitial fibrosis. The present study could not provide a definite explanation for these paradoxical findings, perhaps because total ECM collagen content is a complex function of synthesis and degradation.

Although the functional role of MMP-2 in this aspect and its significance in myocardial inflammation remain unknown, on the basis of a previous study by Heymans et al. (9), we could not exclude the possibility that deletion of the MMP-2 gene might alter ECM components other than collagens, disrupt the alignment of myocytes with the ECM or degrade the ECM surrounding the myocytes, and promote the further progression of inflammatory cell migration into the interstitial space. MMPs have been shown to facilitate inflammatory cell recruitment (7). However, the present study does not provide a direct proof for the cause-and-effect relation between the increase in cellular infiltration and the exacerbation of heart failure, and further investigation is clearly needed.

The results of the present study are consistent with those of previous studies of MMP-2 gene ablation in other models of tissue inflammation (3, 13). Disruption of the MMP-2 gene exacerbated allergic lung inflammation and increased lethal susceptibility to asphyxiation in a mouse model of asthma (3). Furthermore, Itoh et al. (13) demonstrated an increase in severity of arthritis in association with tissue inflammation in

### Table 2. Plasma levels of cytokines and MMPs

<table>
<thead>
<tr>
<th>Cytokines/chemokines</th>
<th>WT/MMP+/+</th>
<th>WT/MMP−/−</th>
<th>TG/MMP+/+</th>
<th>TG/MMP−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/ml</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>9.0±0.2</td>
<td>9.0±0.8</td>
<td>9.0±0.6</td>
<td>10.4±2.5</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>20.7±10.2</td>
<td>8.3±3.2</td>
<td>17.3±3.3</td>
<td>24.9±6.3</td>
</tr>
<tr>
<td>MMP</td>
<td>60.9±3.0</td>
<td>0.9±0.2*</td>
<td>63.2±2.5</td>
<td>0.8±0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5. ND, not detectable; MCP, monocyte chemoattractant protein. *P < 0.01 vs. WT/MMP+/+.
MMP-2 KO mice. These results indicate that MMP-2 suppressed the development of inflammatory disease. Therefore, TNF-α overexpression and MMP-2 deletion might synergistically activate the cellular inflammatory response in the heart, which could further damage cardiac function.

The present study could not demonstrate the long-term beneficial effects of MMP-2 deletion on the decline in LV systolic function in TNF-α-induced cardiomyopathy. These findings are in contrast to those from a previous study, in which broad-spectrum MMP inhibition could ameliorate myocardial dysfunction in TNF-α TG mice (18). The MMP inhibitor batimatostat was found to reduce myocardial hypertrophy and diastolic dysfunction. Even though it is difficult to compare the two studies directly because of the broad-spectrum action of the MMP inhibitor used by Li et al. (18), these studies suggest that MMP-2 has a complex role in maintaining the physiological function of the heart, and information about the action of individual MMPs with the use of such an inhibitor is limited. The most effective way to evaluate the contribution of the specific MMP and obtain direct evidence for a role of MMP in heart failure is through gene manipulation, as employed in the present study. Whatever the mechanisms are, MMP-2 could protect the heart by inhibiting acceleration of interstitial inflammatory infiltration in this model. The disparity between selective and nonselective inhibition of MMP-2 may have important implications in the development of pharmacological agents for the treatment of heart failure. These findings may further draw attention to treatment of heart failure by using the nonselective, broad-spectrum MMP-2 inhibitor.

There are several limitations to be acknowledged in this study. 1) We examined only female mice for physiological, pathological, and biochemical analyses, because most male TG mice died before 12 wk of age, when these analyses were performed. As a result, we are not certain whether selective disruption of the MMP-2 gene exacerbates myocardial inflammation and dysfunction even in male TNF-α TG mice. 2) Even though in vivo assessment of LV function with echocardiography is feasible and reproducible in the mouse, it might be difficult to interpret the indexes in dilated LV. However, our validation study has shown that intra- and interobserver variabilities of our echocardiographic measurements for LV cavity dimensions and fractional shortening were small and that measurements in the same animals on separate days were highly reproducible (24). Therefore, our technique could be considered to allow for a noninvasive assessment of LV structure. 3) The heart rates in the present study (450–470 beats/min) were lower than those measured in conscious mice (600 beats/min). Therefore, the LV size and function results might be greatly influenced by differences in anesthetic regimens and experimental conditions, such as heart rate. 4) Although the present study employed the conventional gelatin zymography method to analyze MMP activities, as in a previous study (8), two-dimensional zymography is essential, especially to ascertain the existence of plasmin in the 66-kDa MMP complex (28). 5) We assessed TGF-β, which has been well established to be involved in ECM remodeling in heart failure, in the present study. Further studies on TGF-α are also needed, because it plays an important role in cell proliferation and differentiation.

In summary, selective disruption of the MMP-2 gene exacerbated myocardial inflammation and dysfunction in TNF-α-induced cardiomyopathy. Thus myocardial expression of MMP-2 may play a protective role in the development of congestive heart failure in cytokine-induced cardiomyopathy.

GRANTS

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REFERENCES


