Inhibition of TGF-β signaling exacerbates early cardiac dysfunction but prevents late remodeling after infarction

Masaki Ikeuchi, Hiroyuki Tsutsui*, Tetsuya Shiomi, Hidenori Matsusaka, Shouji Matsushima, Jing Wen, Toru Kubota, Akira Takeshita

Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi, Fukuoka, 812-8582, Japan

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Abstract

Objective: Transforming growth factor (TGF)-β promotes the deposition of extracellular matrix protein and also acts as an anti-inflammatory cytokine. These biological effects might be involved in the development and progression of left ventricular (LV) remodeling and failure after myocardial infarction (MI). However, its pathophysiological significance remains obscure in post-MI hearts.

Methods: Anterior MI was produced in mice by ligating the left coronary artery. TGF-β mRNA levels increased in both infarcted and noninfarcted LV after MI. To block TGF-β signaling during the early phase of MI, an extracellular domain of TGF-β type II receptor (TβIIIR) plasmid was transfected into the limb skeletal muscles 7 days before ligation.

Results: TβIIIR increased the mortality during 24 h of MI, as well as exacerbated LV dilatation and contractile dysfunction, the infiltration of neutrophils, and gene expression of tumor necrosis factor-α, interleukin-1β, and monocyte chemoattractant protein-1 compared with nontreated MI mice despite the comparable infarct size. Next, to block TGF-β signaling during the later phase, TβIIIR was transfected into mice at days 0 and 7 after ligation. At 4 weeks, LV dilatation and contractile dysfunction in association with myocyte hypertrophy and interstitial fibrosis of noninfarcted LV seen in MI mice were prevented by TβIIIR.

Conclusions: The activation of TGF-β is protective against ischemic myocardial damage during the early phase. However, the beneficial effects might be lost, when its expression is sustained, thereby leading to LV remodeling and failure after MI.

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1. Introduction

Myocardial infarction (MI) leads to complex structural alterations (remodeling) involving both the infarcted and noninfarcted left ventricular (LV) myocardium. Early remodeling is LV cavity dilatation occurring during the early phase of MI, which is likely due to wall thinning of the infarct region. During the first several days, LV enlargement follows, and thereafter, a progressive dilatation of the noninfarcted LV associated with myocyte hypertrophy and interstitial fibrosis occurs over weeks [1]. These progressive changes in LV geometry contribute to the development of depressed cardiac function, clinical heart failure, and increased mortality. Accordingly, it is of critical importance to explore the mechanisms and to develop therapeutic strategies that will effectively inhibit this deleterious process.

Transforming growth factor (TGF)-β is a locally generated cytokine that has been implicated as a major stimulator of tissue fibrosis [2]. It has a major influence on fibroblast proliferation and extracellular matrix (ECM) production, particularly of collagen and fibronectin, while reducing degradation of these components. TGF-β expression has been shown to increase not only during cardiac...
hypertrophy [3], but also in post-MI hearts [4]. These findings raise the hypothesis that TGF-β may adversely affect LV remodeling and failure after MI.

On the contrary, induction of TGF-β expression as well as supplementation with exogenous TGF-β could protect cardiomyocytes against ischemia-reperfusion injury [5]. Cardioprotection by TGF-β has been attributed to the inhibition of tumor necrosis factor-α (TNF-α) and prevention of reactive oxygen species (ROS) generation [6]. TNF-α has been shown to exert cardioprotective effects against myocardial damage [7–9], whereas its excess causes heart failure [10]. The overproduction of ROS is also involved in myocardial remodeling [11]. Based on these findings, it is conceivable to hypothesize that TGF-β may play a protective role against post-MI failure by modulating TNF-α or ROS.

TGF-β, a multifunctional polypeptide, may exert the differential effects on early vs. late process of LV healing and remodeling after MI and play an important role in the pathophysiology of heart failure. Therefore, by the gene transfer technique using an extracellular domain of TGF-β type II receptor plasmid, we determined the effects of TGF-β blockade separately on the early and late phases of post-MI conditions.

2. Methods

2.1. Anti-TGF-β gene therapy

The study was approved by our Institutional Animal Research Committee and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). To block TGF-β1 signaling, plasmids expressing an entire extracellular domain of human TGF-β type II receptor fused to the Fc portion of human IgG, TβIIR, were employed. Previous studies demonstrated that TβIIR secreted from TβIIR plasmid-infected cells could bind to TGF-β and thereby effectively block its signaling in vitro as well as in vivo [12].

Anesthetized mice were injected in the bilateral tibial muscles with either empty or TβIIR plasmid in phosphate-buffered saline using a 27-gauge needle fitted with a plastic collar limiting muscle penetration to approximately 5 mm. To improve the gene transfection efficiency, electroporation was performed immediately after the injection of plasmids.

2.2. Creation of MI

We created MI in male CD-1 mice, 6–8 week old and 30–40 g body weight, by ligating the left coronary artery [13]. In brief, after anesthesia, the endotracheal tube was placed in the trachea and the ventilation was provided by a rodent ventilator. The chest was opened and the left auricle was slightly retracted exposing the entire left coronary artery. An 8–0 silk suture was passed underneath the left anterior descending coronary artery 1–3 mm from tip of the normally positioned left auricle and the coronary ligation was performed. The chest wall was then closed, and the animal was kept warm by a heat lamp and allowed 100% oxygen via nasal cone for recovery.

2.3. Experimental protocols

Our preliminary studies demonstrated that serum levels of TβIIR protein, determined using ELISA with rabbit anti-human IgG antibody, peaked from <20 to 130–140 ng/ml 7–10 days after a single injection of plasmid (3 μg/g body weight) and gradually decreased thereafter (Fig. 1). These time-dependent changes of TβIIR could allow us to determine the effects of TGF-β blockade separately on the early and late phases of post-MI conditions by altering the timing of injection. To determine the effects of TβIIR on post-MI remodeling, we performed two substudies (Fig. 2). In the substudy 1, TβIIR was transfected 7 days before ligation and the effects of TGF-β blockade on the early LV dysfunction were examined at day 1. In the substudy 2, TβIIR was transfected at days 0 and 7 and LV structure and function were examined at day 28.

2.4. Substudy 1: early (within 24 h) post-MI phase

Substudy 1 consisted of four groups of animals; sham+vehicle (n=22), sham+TβIIR (n=22), MI+vehicle (n=31), and MI+TβIIR (n=27).

2.4.1. Survival

The survival analysis was performed in the MI+vehicle and MI+TβIIR mice. During the study period of 24 h, cages were inspected for deceased animals. All deceased mice...
were examined for the presence of MI as well as pleural effusion and cardiac rupture.

2.4.2. Echocardiographic and hemodynamic measurements

At 9 h after MI, echocardiographic studies were performed under light anesthesia with tribromoethanol/amylene hydrate (Avertin; 2.5% w/v, 8 µl/g i.p.) and spontaneous respiration as described previously [13]. Echocardiographic studies were performed by using an ultrasonograph (SSD-5500, Aloka, Tokyo). A dynamically focused 13-MHz annular array ultrasound transducer was placed over the chest. Then two-dimensionally directed M-mode of the LV minor axis was taken at the papillary muscle level. Our previous study has shown that the intraobserver and interobserver variabilities of our echocardiographic measurements for LV dimensions were small and measurements made in the same animals on separate days were highly reproducible [13]. Then, a 1.4-Fr micromanometer-tipped catheter (Millar) was inserted into the right carotid artery and then advanced into the left ventricle to measure LV pressures.

2.4.3. Risk area and infarct size

To evaluate the effects of Tβ3IIR on the risk area and infarct size, a separate group of animals including MI+vehicle (n=7) and MI+Tβ3IIR (n=7) was created. After 9 h, mice were anesthetized with tribromoethanol/amylene hydrate. Evans blue dye (1%) was perfused into the aorta and coronary arteries with distribution throughout the LV wall proximal to the site of coronary artery ligation according to the methods described by Michael et al. [14]. The non-ischemic area was stained blue. Hearts were excised and sliced into cross sections below the ligature. These sections were weighed and then incubated with a 1% triphenyltetrazolium chloride (TTC) solution at 37°C for 20 min. The infarct area (pale), the area at risk (not blue), and the total LV area from each section were measured, multiplied by the weight of the section, and then totaled from all sections.

2.4.4. Myocardial histopathology

After in vivo hemodynamic studies performed in the survived animals, the heart was excised and dissected into the right and left ventricles, including the septum. The LV was cut into three transverse sections: apex, middle ring, and base. Five-micrometer sections were cut and stained with Masson’s trichrome and Sirius red. Myocyte cross-sectional area and collagen volume fraction were determined by quantitative morphometry of LV tissue sections [13]. For the morphometric analysis, each section was photographed using a microscope and magnified. Eight fields (210×170 µm² for each field) were randomly selected from two to three coronal sections of each mouse. Connective tissue and muscle areas were identified and the profile margin of myocytes cut into cross sections was manually traced and digitized. The digitized profiles were transferred to a personal computer that calculated the area.

To determine the numbers of infiltrating polymorphonuclear neutrophils, the tissue sections were also stained with hematoxyline and eosin and the numbers of neutrophils per whole LV section were counted.

2.4.5. Myeloperoxidase (MPO) activity

Ribonuclease protection assay was performed with 5 µg of total RNA isolated from LV to determine the expression level of genes including TGF-β, regulated on activation, normal T cell expressed and secreted (RANTES), TNF-α, interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1). Each value was normalized to that of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) in each template set as an internal control.

2.5. Substudy 2: late (after 28 days) post-MI phase

Substudy 2 consisted of four groups of animals: sham+vehicle (n=20), sham+Tβ3IIR (n=20), MI+vehicle (n=26), and MI+Tβ3IIR (n=26). The surgical procedure and the experimental protocols were identical to those in substudy 1. Infarct size was measured by the morphometric analysis of LV sections [13]. As an index of reactive oxygen species (ROS) production, the degree of lipid peroxidation was determined through biochemical assay of thiobarbituric acid reactive substances (TBARS). Endothelial and inducible nitric oxide synthase (eNOS and iNOS, respectively) protein levels were measured by Western blot analysis with specific antibodies.
2.6. Statistical analysis

Data are expressed as means±SEM. Survival analysis was performed by the Kaplan–Meier method, and between-group difference in survival was tested by the log-rank test. Between-group comparison of means was performed by one-way ANOVA, followed by t-tests. The Bonferroni’s correction was done for multiple comparisons of means.

3. Results

3.1. TGF-β gene expression after MI

TGF-β mRNA levels were significantly elevated in both infarcted and noninfarcted LV after MI (Fig. 3). TGF-β was elevated in the infarcted LV from day 1 to 28 with a peak value at 7 days. In the noninfarcted LV, TGF-β remained elevated until day 28 with a peak at day 3.

3.2. Substudy 1: early post-MI phase

3.2.1. Mortality

The mortality rate up to 24 h of MI was significantly higher in MI+TβIIR compared to MI+vehicle (53% vs. 19%, P<0.01). No mice died from LV rupture. There were no deaths in sham-operated groups.

3.2.2. Risk area and infarct size

Percentages of LV at risk (risk area/LV; 60±1% vs. 60±1%, P=NS) and the infarct size (infarct/risk area; 69±2% vs. 71±3%, P=NS) measured after 9 h of MI were comparable between MI+vehicle and MI+TβIIR hearts, suggesting that higher mortality was not attributable to the differences in infarct size between groups. Importantly, the mortality was comparable between MI+vehicle and MI+TβIIR (13% vs. 22%; P=NS) in this measurement, indicating that the selection bias due to the earlier death in animals with larger infarct is less likely.

3.2.3. LV function and structure

Analysis of LV function and structure after 9 h of surgery demonstrated that there was no significant difference in heart rate among four groups. LV fractional shortening was decreased in both MI+vehicle and MI+TβIIR groups and TβIIR significantly exacerbated LV dysfunction caused by MI (Fig. 4A and Table 1).

Mean aortic blood pressure was lower in MI compared to sham, which, however, did not differ between MI+vehicle and MI+TβIIR. LV end-diastolic pressure (EDP) was increased in MI+vehicle, which was further increased in MI+TβIIR. Coinciding with an increased LVEDP, lung weight/body weight was increased in the MI groups, which was further elevated by TβIIR. The prevalence of pleural effusion was significantly higher in TβIIR-transfected MI mice. These results suggested that TβIIR exacerbated LV dysfunction in MI, which might lead to higher mortality in MI+TβIIR group.

3.2.4. Myocardial pathology and MPO activity

Myocyte cross-sectional area (175±7 vs. 178±5 μm², P=NS) and collagen volume fraction (1.6±0.3% vs. 1.5±0.2%, P=NS) measured in the infarcted LV were comparable between MI+vehicle and MI+TβIIR after 9 h. Myocyte cross-sectional area was 173±3 μm² and collagen content was 1.6±0.3% in sham+vehicle, which did not differ from MI values.

![A Infarcted LV](image1)

![B Noninfarcted LV](image2)

Fig. 3. Time-dependent changes of TGF-β mRNA levels in the infarcted (A) and noninfarcted LV (B) (n=9 for each time point). Values are means±S.E.M. *P<0.05, **P<0.01 for difference from the sham values.
The infiltration of neutrophils was barely detectable within the LV from sham-operated animals. In contrast, its number increased in the infarcted LV obtained from MI, which was further increased by T\(\beta\)IIIR (Fig. 5A and B). Neutrophils were not detected in the noninfarcted LV and they were not altered by T\(\beta\)IIIR. In accordance with neutrophil counts, MPO activity was significantly increased in the infarcted LV from MI+T\(\beta\)IIIR (Fig. 5C). The increase in the neutrophil counts within the infarcted LV by T\(\beta\)IIIR might be attributable to higher mortality in MI+T\(\beta\)IIIR group.

Table 1

<table>
<thead>
<tr>
<th>Echocardiographic data</th>
<th>Sham+vehicle</th>
<th>Sham+T(\beta)IIIR</th>
<th>MI+vehicle</th>
<th>MI+T(\beta)IIIR</th>
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<tr>
<td>(N)</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>472±13</td>
<td>461±12</td>
<td>487±9</td>
<td>497±8</td>
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<td>LVEDD (mm)</td>
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<td>LVESD (mm)</td>
<td>2.74±0.04</td>
<td>2.70±0.04</td>
<td>3.48±0.04**</td>
<td>3.80±0.04**(\text{a})</td>
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<td>Fractional shortening (%)</td>
<td>35.9±0.3</td>
<td>36.3±0.5</td>
<td>21.0±0.2**</td>
<td>14.8±0.3**(\text{a})</td>
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<tr>
<td>Infarct wall thickness (mm)</td>
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<td>–</td>
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<td>0.62±0.02</td>
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<tr>
<td>Noninfarct wall thickness (mm)</td>
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<th>Hemodynamic data</th>
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<tr>
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<td>20</td>
<td>22</td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>467±11</td>
<td>460±11</td>
<td>473±7</td>
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<tr>
<td>Mean aortic pressure (mm Hg)</td>
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<td>79±2</td>
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<td>60±2**</td>
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<td>LVEDP (mm Hg)</td>
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<td>1.4±0.4</td>
<td>16.1±0.9**</td>
<td>20.6±1.0**(\text{a})</td>
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<th>Organ weights</th>
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<th>MI+vehicle</th>
<th>MI+T(\beta)IIIR</th>
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<td>(N)</td>
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<td>22</td>
<td>15</td>
<td>17</td>
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<tr>
<td>Body wt (g)</td>
<td>34.5±0.5</td>
<td>34.6±0.2</td>
<td>34.3±0.6</td>
<td>35.2±0.5</td>
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<td>LV wt/Breast wt (mg/g)</td>
<td>2.8±0.1</td>
<td>2.8±0.1</td>
<td>3.1±0.1</td>
<td>2.9±0.1</td>
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<tr>
<td>RV wt/Breast wt (mg/g)</td>
<td>0.85±0.05</td>
<td>0.86±0.03</td>
<td>0.75±0.04</td>
<td>0.70±0.04</td>
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<tr>
<td>Lung wt/Breast wt (mg/g)</td>
<td>5.9±0.1</td>
<td>5.9±0.1</td>
<td>7.2±0.2**</td>
<td>8.7±0.3**(\text{a})</td>
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<tr>
<td>Pleural effusion (%)</td>
<td>0</td>
<td>0%</td>
<td>47</td>
<td>82</td>
</tr>
</tbody>
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MI, myocardial infarction; LV, left ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; EDP, end-diastolic pressure. wt, weight; RV, right ventricular. Values are means±S.E.M.

\(\text{** } P<0.01\text{ vs. sham+vehicle.}\)

\(\text{\(\text{a}\) } P<0.01\text{ vs. MI+vehicle.}\)
3.2.5. Cytokines

RANTES, TNF-α, IL-6, IL-1β, and MCP-1 were significantly increased in the infarcted LV after 9 h. TβIIR further enhanced TNF-α, IL-1β, and MCP-1 genes (Fig. 6A and B). Similar to infarcted LV, cytokine genes were increased also in noninfarcted LV, which, however, were not affected by TβIIR (Fig. 6A).

![Figure 5](image_url)

**Fig. 5.** (A) Photomicrographs of hematoxyline-eosin-stained LV cross section in the infarct area obtained from MI+vehicle and MI+TβIIR mice 9 h of MI. Arrows indicate the infiltrating neutrophils. Scale bar, 10 μm. (B) Neutrophil counts in the infarct area from MI+vehicle (n=6) and MI+TβIIR (n=6) mice. (C) MPO activity in the infarcted and noninfarcted LV from MI+vehicle (n=6) and MI+TβIIR (n=6) mice. *P<0.05 for difference from the MI+vehicle value.

![Figure 6](image_url)

**Fig. 6.** (A) Cytokine gene expression from sham+vehicle, sham+TβIIR, MI+vehicle, and MI+TβIIR mice after 9 h. It was analyzed in the infarcted and noninfarcted LV from MI groups. (B) Neutrophil counts in the infarct area from MI+vehicle (n=6) and MI+TβIIR (n=6) mice. (C) MPO activity in the infarcted and noninfarcted LV from MI+vehicle (n=6) and MI+TβIIR (n=6) mice. *P<0.05 for difference from the MI+vehicle value.
3.3. Substudy 2: late post-MI phase

3.3.1. Survival

The survival rate up to 4 weeks was not significantly different between MI+vehicle and MI+TβIIR (50% vs. 64%, P=NS). Four MI+vehicle (15%) and one TβIIR-treated MI (4%) mice died from LV rupture (P=NS). There were no deaths in sham-operated groups.

3.3.2. LV function and structure

Echocardiographic studies at 28 days demonstrated that TβIIR transfection significantly attenuated LV dilatation...
and dysfunction (Fig. 4B and Table 2). In comparison with sham, MI animals showed a significant increase in the thickness of noninfarcted LV, which was attenuated by TβIIR.

LVEDP was increased in MI+vehicle, which was attenuated in TβIIR-treated MI mice. TβIIR partially normalized LV dP/dt max and dP/dt min, which were significantly reduced in MI+vehicle.

Right ventricular weight/body weight was increased in MI and TβIIR attenuated this increase. Coinciding with an increased LVEDP, lung weight/body weight was increased in the MI groups, which was also attenuated by TβIIR. The prevalence of pleural effusion was significantly lower in MI+TβIIR.

### 3.3.3. Infarct size and myocardial pathology

Infarct size was comparable (53±2% vs. 53±2%; P=NS) between MI+vehicle (n=8) and MI+TβIIR (n=8) mice.

The collagen volume fraction was increased in MI compared to sham, which was inhibited by TβIIR transfection (Fig. 7). Myocyte cross-sectional area was also increased in MI compared to sham, which was attenuated by TβIIR (Fig. 7). These results are concordant with LV wall thickness data obtained from echocardiography (Table 2). Both myocyte cross-sectional area and collagen volume fraction were increased in the noninfarcted LV from MI after 28 days compared to those after 9 h.

### 3.3.4. Cytokines, ROS, and NOS

Cytokines including RANTES, TNF-α, IL-1β, IL-6, and MCP-1 were increased in the infarcted and noninfarcted LV obtained from MI compared to sham (Fig. 8). When comparing the gene expression between 28 days and 9 h, the extent of increase in IL-6, IL-1β, and MCP-1 genes was smaller in 28 days compared to 9 h of MI (Figs. 6 and 8). Importantly, in contrast to 9 h of MI (Fig. 6), this increase was not significantly altered by TβIIR in 28 days (Fig. 8).

TBARS tended to be lower in the noninfarcted LV from MI+TβIIR compared to MI+vehicle (61±5 nmol/g for n=8 vs. 73±6 nmol/g for n=8), which, however, did not reach statistical significance (P=0.05).

eNOS protein levels were comparable between MI+vehicle and MI+TβIIR (ratio to sham+vehicle value: 1.4±0.2 for n=6 vs. 1.4±0.2 for n=6, P=NS). iNOS protein levels were increased in the MI groups, which, however, were not altered by TβIIR (ratio to sham+vehicle value: 2.1±0.2 for n=8 vs. 2.4±0.2 for n=8, P=NS).

### 4. Discussion

The important and unique findings of this study were twofold. First, TGF-β inhibition in the early post-MI period exacerbated the degree of LV contractile dysfunction, which might be related to an increased extent of inflammatory process in the infarct area. Second, during the later phase, TGF-β inhibition attenuated LV hypertrophy and interstitial fibrosis, thereby progressive LV remodeling and failure. Thus, these results demonstrate that initial activation of TGF-β after MI is protective against ischemic myocardial dysfunction, but its activation produces LV structural remodeling, which leads to deleterious cardiac failure.
TGF-β was increased in both infarcted and non-infarcted LV early after MI and its increase persisted thereafter (Fig. 3), which is in agreement with previous studies [4]. Because TGF-β is one of growth factors that influence tissue repair and remodeling, its activation might be well expected to play an important role in LV structural changes after MI.

A well-defined temporal sequence of cellular and extracellular events occurs after acute MI and determines the initial wound-healing response. This early phase of post-MI healing is associated with neutrophil infiltration within the infarcted area. The present study demonstrated that early abrogation of TGF-β signaling exaggerated LV dysfunction in association with an increase in the number of neutrophils (Fig. 5). Even though the neutrophil counting on the tissue sections stained with hematoxyline and eosin underestimates their number and a better way would have been the histochemical staining such as naphthol AS-D chloroacetate esterase [15], our morphometric data are supported by the biochemical measures of MPO activity. In addition, the inhibition of TGF-β signaling enhanced the gene expression of such cytokines as TNF-α, IL-1β, and MCP-1 (Fig. 6). Therefore, TGF-β may play a protective role against early myocardial failure after MI by modulating the cytokine gene expression. These results are consistent with previous studies that TGF-β can protect the heart against ischemia-reperfusion injury via inhibiting TNF-α [6].

Previous studies have suggested that TGF-β might be involved in cardiac hypertrophy and fibrosis [16]. However, it is not known whether there is a causal relationship between increased TGF-β and these cardiac pathologies. The present study clearly demonstrated that long-term TGF-β inhibition decreased myocyte hypertrophy and interstitial fibrosis. Thus, TGF-β is involved in the pathogenesis of LV remodeling not only under pressure overload [3], but also in the post-MI conditions. The beneficial effects of TβIIR were not due to its MI size-sparing effect. Further, its effects might not be attributable to those on hemodynamics. TGF-β is capable to stimulate the proliferation of mesenchymal cells and the production of ECM components in a variety of cell types [17]. Previous studies of Petrov et al. [18] and Lijnen et al. [19] have shown that TGF-β can increase the differentiation of cardiac fibroblasts into myofibroblasts. Therefore, even though no direct evidence was provided in the present study, we speculate that the effects of TβIIR might be due to the modulation of this phenotypic conversion, which plays a major role in tissue fibrosis and organ remodeling by actively producing ECM components as well as profibrotic mediators. Importantly, the inhibition of TGF-β did not induce cardiac rupture, suggesting that TβIIR might not impair the early healing process of infarct tissue.

The triggers responsible for TGF-β induction after MI remain undetermined in this study. Mechanical stress and various cytokines including angiotensin II and TNF-α are supposed to be involved in the pathogenesis of post-MI remodeling, all of which can induce and activate TGF-β [20]. In the setting of acute MI and/or ischemia reperfusion, the expression of TGF-β can protect the heart from the injury and is likely to be beneficial. Early activation of TGF-β after MI may be also important as an early adaptive response to the loss of stroke volume by stimulating the fibroblast proliferation, leading to effective repair and scar formation. However, its sustained expression after MI could induce interstitial fibrosis that leads to inadvertent maladaptive cardiac remodeling. Thus, the beneficial effects of TGF-β might be lost when its expression is sustained after MI.

TGF-β could protect the heart from the ischemia-reperfusion injury by inhibiting the activation of iNOS induced by cytokines and also by ameliorating the decrease in eNOS [21,22]. However, such mechanisms may not play a major role in the cardioprotective effects of TGF-β against post-infarct LV failure in the present study.

There are several methodological issues to be acknowledged in this study. First, the heart rate values in the present study (480 bpm) were lower than those (600 bpm) measured in conscious mice. Therefore, it should be cautious that LV size and function results might be greatly influenced by the differences in anesthetic regimens and the experimental conditions such as heart rate. Nevertheless, our fractional shortening values (36–38%) are similar to those (41–42% at heart rate of 410–420 bpm) in anesthetized mice reported by others [23]. Second, the risk area and infarct size were evaluated in a separate group of animals which were not involved in the main study. Therefore, the values obtained in these groups of animals cannot be extrapolated to the others. However, we have extensively used this animal model and obtained consistent results in our previous studies [24]. Therefore, we consider that these results of infarct size measurements are valid. Third, the blockade of TGF-β signaling by intramuscular injection of TβIIR plasmid is simple and appears to be nontoxic and safe. However, the method to eliminate its expression is not established.

In conclusion, the activation of TGF-β, even though protective against ischemic damage during the early phase of MI, might eventually lead to heart failure during the later phase. Our findings may provide a better understanding of the mechanism(s) of LV remodeling and new insight into the development of novel gene therapeutic strategies in heart failure.

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