Increased Expression of Syndecan-1 Protects Against Cardiac Dilatation and Dysfunction After Myocardial Infarction

Davy Vanhoutte, MS*; Mark W.M. Schellings, MS*; Martin Götte, MS, PhD; Melissa Swinnen, MS; Veronica Herias, PhD; Martin K. Wild, PhD; Dietmar Vestweber, PhD; Emmanuel Chorianopoulos, MD, PhD; Víctor Cortés, MS; Attilio Rigotti, MD, PhD; Mary-Ann Stepp, PhD; Frans Van de Werf, MD, PhD; Peter Carmeliet, MD, PhD; Yigal M. Pinto, MD, PhD; Stephane Heymans, MD, PhD

Background—The cell-associated proteoglycan syndecan-1 (Synd1) closely regulates inflammation and cell-matrix interactions during wound healing and tumorigenesis. The present study investigated whether Synd1 may also regulate cardiac inflammation, matrix remodeling, and function after myocardial infarction (MI).

Methods and Results—First, we showed increased protein and mRNA expression of Synd1 from 24 hours on, reaching its maximum at 7 days after MI and declining thereafter. Targeted deletion of Synd1 resulted in increased inflammation and accelerated, yet functionally adverse, infarct healing after MI. In concordance, adenoviral gene expression of Synd1 protected against exaggerated inflammation after MI, mainly by reducing transendothelial adhesion and migration of leukocytes, as shown in vitro. Increased inflammation in the absence of Synd1 resulted in increased monocyte chemoattractant protein-1 expression, increased activity of matrix metalloproteinase-2 and -9, and decreased activity of tissue transglutaminase, associated with increased collagen fragmentation and disorganization. Exaggerated inflammation and adverse matrix remodeling in the absence of Synd1 increased cardiac dilatation and impaired systolic function, whereas gene overexpression of Synd1 reduced inflammation and protected against cardiac dilatation and failure.

Conclusions—Increased expression of Synd1 in the infarct protects against exaggerated inflammation and adverse infarct healing, thereby reducing cardiac dilatation and dysfunction after MI in mice. (Circulation. 2007;115:475-482.)

Key Words: gene therapy ■ heart failure ■ inflammation ■ myocardial infarction ■ remodeling

Syndecan-1 (Synd1) is a member of a conserved family of 4 heparan and chondroitin sulfate–carrying transmembrane proteins (Synd1, 2, 3, and 4). Synd1 is emerging as a central regulator of inflammation and matrix remodeling during wound healing,1,2 infection,3 and tumor formation4 (reviewed elsewhere5–7). Well-orchestrated interactions between inflammation, extracellular matrix components, and cytokines are essential for normal cardiac healing and functional recovery after myocardial infarction (MI). However, clear evidence on a specific role for Synd1 after MI is lacking.

Clinical Perspective p 482

Expression of Synd1 is low in normal cardiac tissue, but its expression reappears at high levels after MI in rats8,9 and mice.10 Considering an essential role of Synd1 for proper wound healing,5–7 we hypothesized that increased expression of Synd1 after MI may regulate inflammation and normal infarct healing after MI. We postulated that the absence of Synd1 would increase inflammation, which in turn results in increased activity of matrix metalloproteinases (MMPs). Increased MMP activity favors degradation of collagens11 and decreases cross-linking of collagens by inactivation of tissue transglutaminase (tTG),12 both resulting in increased cardiac dilatation.13–16 To address these issues, we investigated infarct healing and cardiac function in Synd1 gene–inactivated mice and after adenoviral Synd1 gene overexpression.

Our data reveal that loss of Synd1 resulted in exaggerated inflammation, collagen disorganization, and increased car-

Received June 8, 2006; accepted November 20, 2006.
From Experimental and Molecular Cardiology/CARIM, University of Maastricht, Maastricht, the Netherlands (M.W.M.S., M.S., V.H., Y.M.P., S.H.); Molecular and Vascular Biology and Center for Transgene Technology and Gene Therapy, VIB (D. Vanhoutte, E.C., P.C.) and Department of Cardiology (F.V.d.W.), University of Leuven, Leuven, Belgium; Department of Obstetrics and Gynecology, Muenster University Hospital, Muenster, Germany (M.G.); Max Planck Institute for Molecular Biomedicine, c/o Institute of Cell Biology, ZMBE, Muenster, Germany (M.K.W., D. Vestweber); Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica, Marcoleta, Santiago, Chile (V.C., A.R.); and George Washington University Medical Center, Washington, DC (M.-A.S.).
*The first 2 authors contributed equally to this article.
The online-only Data Supplement, consisting of expanded materials and data, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.644609/DC1. Correspondence to Stephane Heymans, MD, PhD, Experimental and Molecular Cardiology/CARIM, Department of Cardiology, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail s.heymans@cardio.unimaas.nl © 2007 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.106.644609
diac dilatation and failure after MI. In line with these findings, overexpression of Synd1 protected against adverse infarct remodeling and cardiac failure after MI, indicating a potential therapeutic benefit of increased Synd1.

### Methods

#### Transgenic Mice and Model of MI
The present study was approved by our Institutional Animal Research Committee, and all experiments were performed according to the official rules formulated in the Dutch and German laws on care and use of experimental animals. We used 10- to 14-week-old male and female inbred BALB/c Synd1 knockout (KO) (backcrossed for 11 generations) and their wild-type (WT) littermates weighing 20 to 30 g. Synd1 mice were a gift from M.A. Stepp, PhD, Washington, DC.

MI was induced by permanent ligation of the left coronary artery.17 Sham operation included all procedures except permanent ligation. Hearts were taken out at indicated time points and prepared for molecular, histological, and ultrastructural analyses.17,18 Perioperative mortality rates (24 hours) were 23% and 25% in WT and Synd1 KO mice, respectively (P = NS).

#### Histology and Electron Microscopy of Infarcts

After the study period, infarcted or sham-operated mice were anesthetized, and hearts were removed and prepared for further histological and molecular analyses. Lungs and the left and right ventricles were dissected, blotted dry, and weighed.18 Immunostaining on paraffin sections was performed with antibodies against CD31 (capillaries), CD45 (leukocytes), α-smooth muscle cell actin (coronary vessels) (Dako, Leuven, Belgium), e-lysyl γ-glutamylaminyl cross-links (Abcam, Cambridge, Mass), and Synd1 (CD138; Pharmingen, Erembodegem, Belgium) as previously described.17,18 CD31-staining capillaries, CD45-staining leukocytes, and smooth muscle cell actin–staining vessels and myofibroblasts were counted and quantified in the different areas through the infarcted left ventricle as described.17,18 Morphometric analysis was performed with a Leitz DMRXE microscope (Leica Imaging Systems Ltd, Solms, Germany), a 3 CCD color video camera (DXC-930P, Sony, New York, NY), and a Leica Qwin software system by persons unaware of the genotype.

#### Syndecan Expression

First, in situ hybridization of Synd1 mRNA was performed on cardiac sections at 1, 4, 7, and 14 days after MI (online Data Supplement, Section I). Synd1 mRNA-staining cells were counted per square millimeter. Next, Synd1 dot-blot analysis was performed in cardiac extracts with determination of total and shed protein of Synd1.2 Finally, to investigate whether absence of Synd1 may result in a compensatory increase in other syndecans, mRNA levels of Synd1, 2, 3, and 4 were determined by real-time polymerase chain reaction with qSYBR green supermix (Bio-Rad Laboratories, Hercules, Calif) in infarcts and sham heart at 7 days after MI (online Data Supplement, Section II).

#### In Vitro Leukocyte Adhesion and Transmigration Assays

To obtain polymorphonuclear cells (PMNs), bone marrow was flushed out of the hindlimbs of Synd1 KO and WT mice with PBS/1% fetal calf serum, and PMNs were purified on Histopaque 1077/1119 gradients (Sigma, Deisenhofen, Germany). Purity of the preparation was controlled by flow cytometry or cytospin.19

Static assays measuring the adhesion of PMNs to the murine bEnd.3 endothelial cell line were performed essentially as described.19 In short, PMNs were fluorescently labeled with 2′,7′-bis-(2-carboxyethyl)-5-carboxyfluorescein acetoxymethyl ester (Molecular Probes, Eugene, Ore), washed, and incubated in triplicates to sexuplets (2×10⁶ cells/mL, 50 μL/well) with confluent bEnd.3 monolayers in 96-well plates (10 minutes, 37°C). After washing with PBS, adhering cells were lysed.19 The fluorescence signal was quantified in a Spectramax (MTX Lab Systems, Inc, Vienna, Va) fluorimeter (excitation, 485 nm; emission, 535 nm).

For transendothelial migration assays, 5×10⁹ PMNs in RPMI 1640 medium containing 0.5% BSA were added in triplicate to bEnd.3 cells grown for 2 days in laminin-coated 6.5-mm Transwells (5-μm pore size, Costar, Bodenheim, Germany). The lower chambers contained Dulbecco’s modified Eagle’s medium/10% fetal calf serum. bEnd.3 cells were stimulated for 16 hours with 5 μmol/L human tumor necrosis factor-α (R&D Systems, Wiesbaden, Germany) before PMNs were added. Transmigrating PMNs were counted in a CASY cell counter (Schärfe System, Reutlingen, Germany).

#### Transcript Levels of Cytokines In Vivo

Transcript levels of the cytokines monocyte chemoattractant protein-1; regulated-on-activation, normal T-cell–expressed and –secreted chemokine; tumor necrosis factor-α; and stromal cell–derived factor were investigated in infarcted and sham tissue of Synd1 KO compared with WT mice with acidic ribosomal phosphoprotein P0 (ARBP) as the housekeeping gene. Methodology and a list of the primers used are presented in the online Data Supplement, Section II.

#### Collagen Content

First, transcript levels of collagen type I and III were investigated in infarcted and sham hearts of Synd1 KO compared with WT mice (online Data Supplement, Section II). Next, collagen was stained on cardiac sections with Sirius red, and the amount of collagen was measured by real-time polymerase chain reaction (online Data Supplement, Section II) and immunoblottting (ab2972-500, Abcam).

#### tTG and MMP Activity in Infarcts

Zymographic activity of MMP-2 and -9 in infarcted and sham hearts was performed as described.17,22

Because MMP-2 is able to inactivate tTG, activity of tTG in the infarcted area was measured by incorporating biotinylated cadaverine into fibronectin.12 Next, transcript levels and protein expression of tTG were measured by real-time polymerase chain reaction (online Data Supplement, Section II) and immunoblottting (ab2972-500, Abcam).

#### Adenoviral Gene Transfer of Synd1

Replication-deficient adenoviruses containing rat Synd1 cDNA (AdSynd1) or the control R5 gene (AdR5), both under control of the cytomegalovirus promoter, were generated by homologous DNA recombination in bacteria, followed by viral particle generation and large-scale production in HEK293 cells as described.23

Then, 100 μL of 1.0×10⁹ plaque-forming units AdSynd1 or control AdR5 diluted in saline was injected into the tail vein of Synd1 WT mice, followed by induction of MI. Synd1 plasma levels were semiquantitatively measured in 100 μL blood sampled from the retro-orbital plexus by Synd1 immunoblotting (Sc-5632, Santa Cruz Biotechnology, Santa Cruz, Calif) at 7 days after gene transfer. Echocardiography was performed in Synd1 and control R5-treated at 14 days after MI, followed by histological and molecular analyses of infarcted hearts.

#### Echocardiographic Measurements

Transhorrect echocardiography with a 12-MHz probe (Hewlett Packard, Amsterdam, the Netherlands) on a Sonos 5500 echocardiograph (Hewlett Packard, Palo Alto, Calif) was performed as described before.17,22
Results

Increased Expression of Synd1 After MI

In situ hybridization of Synd1 revealed minimal Synd1 mRNA expression in the infarcted myocardium of WT mice at 1 day after MI (n=7), with a progressive increase in Synd1 mRNA from 4 days (n=6) (Figure 1A), reaching its maximum at 7 days (n=8) (Figure 1A and 1B) and decreasing again at 14 days after MI (n=10) (Figure 1A). Synd1 mRNA was absent in sham-operated hearts. Expression was located primarily in inflammatory cells, endothelial cells, and (myo) fibroblasts in the infarcted and peri-infarct region.

Concordant with in situ hybridization, Synd1 immunostaining and dot-blot analysis revealed significantly increased expression of Synd1 in the infarct region, with a maximal expression at day 7, again declining at day 14 (Figure 1C through 1E). Ratio of shed to total Synd1 increased significantly in the infarcted myocardium at 1 and 4 days but declined at 7 and 14 days compared with sham levels (Figure 1F).

To investigate whether the absence of Synd1 may result in compensatory changes in expression of other syndecans, transcript levels of Synd1, 2, 3, and 4 were determined in Synd1 KO compared with WT heart at 7 days after MI or sham surgery. Transcript levels of Synd2, 3, and 4 were significantly increased in WT and KO infarcts at 7 days after MI compared with sham hearts, concordant with previous findings by Finsen et al10 (Table 1). Lack of Synd1, however, did not result in a compensatory increase in Synd2, 3, or 4 after MI, nor did levels of Synd2, 3, or 4 differ in sham hearts of Synd1 KO compared with WT mice (Table 1).

Increased Recruitment of Inflammatory Cells in Synd1 KO Mice

Infarct size did not differ significantly between Synd1 WT and KO mice (Table 2). The number of CD45-immunoreactive inflammatory cells (Figure 2C and 2D, Table 2) was significantly increased in Synd1 KO compared with WT infarcts at 7 and 14 days. Increased inflammation in the absence of Synd1 resulted in accelerated replacement of injured cardiomyocytes by granulation tissue, as indicated by significantly decreased amounts of residual myocardial necrosis, increased smooth muscle cell actin–immunoreactive myofibroblasts and coronary vessels, and increased CD31-immunoreactive capillaries in Synd1 KO compared with WT infarcts at 7 days after MI (Table 2 and Figure 2A and 2B).

Increased inflammation in the infarct was associated with significantly increased transcript levels of monocyte chemotactic protein-1 (Table 1), involved in adverse infarct healing and increased cardiac dilatation and dysfunction after MI.24,25 Increased inflammation in Synd1 KO infarct was related to significantly increased adhesion and transmigration of PMNs through bEnd.3 murine endothelial cells in vitro in Synd1 KO compared with WT PMNs (adhesion, n=6, \( P<0.05 \); transmigration, n=4, \( P<0.001 \)) (Figure 3A through 3D).

Together, these data show that lack of Synd1 results in increased influx of inflammatory cells in the infarcted myocardium with accelerated wound healing and reveal a direct role for Synd1 in regulating transendothelial adhesion and transmigration of inflammatory cells in vitro.

Deficient Collagen Maturation and Organization in the Absence of Synd1

Next, we investigated whether accelerated infarct healing in absence of Synd1 affected collagen remodeling. Transcript
levels of collagen type Iα1 and III and amount of collagen were significantly increased in Synd1 KO compared with WT infarcts but did not differ between sham-operated hearts (Figure 4A and 4B, Tables 1 and 2). Sirius red polarization microscopy revealed mainly well-aligned and thick, tightly packed (orange-red) collagen fibers in WT infarcts (Figure 4C). In contrast, loosely assembled (yellow-green) collagen fibers predominated in Synd1 KO infarcts at 14 days (ratio of orange-red to yellow-green birefringent collagen in the infarct area at 14 days; Figure 4C and 4D, Table 2). Ultrastructural analysis confirmed a disorganized matrix with a predominance of smaller and fragmented collagen fibers in Synd1 KO infarcts, in contrast to the well-organized collagen matrix with uniform and sharply delineated collagen fibers in WT infarcts (Figure 4E and 4F).

Thus, increased inflammation and accelerated infarct healing in the absence of Synd1 resulted in increased collagen deposition, but of impaired quality.

**TABLE 1. Transcript Levels of Syndecans, Cytokines, and Collagens After MI**

<table>
<thead>
<tr>
<th></th>
<th>Synd1 WT, Sham (n=4)</th>
<th>Synd1 KO, Sham (n=5)</th>
<th>Synd1 WT, MI (n=7)</th>
<th>Synd1 KO, MI (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI, 7 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synd1</td>
<td>1.6±0.13</td>
<td>ND</td>
<td>14±1.3†</td>
<td>ND</td>
</tr>
<tr>
<td>Synd2</td>
<td>0.40±0.02</td>
<td>0.35±0.06</td>
<td>0.96±0.08†</td>
<td>0.92±0.10†</td>
</tr>
<tr>
<td>Synd3</td>
<td>0.48±0.03</td>
<td>0.39±0.03</td>
<td>1.1±0.17†</td>
<td>0.73±0.07†</td>
</tr>
<tr>
<td>Synd4</td>
<td>0.43±0.05</td>
<td>0.39±0.06</td>
<td>1.2±0.12†</td>
<td>1.0±0.16†</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.06±0.01</td>
<td>0.09±0.03</td>
<td>0.36±0.08†</td>
<td>0.77±0.15†</td>
</tr>
<tr>
<td>RANTES</td>
<td>0.17±0.02</td>
<td>0.18±0.03</td>
<td>1.6±0.45†</td>
<td>0.80±0.16†</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.10±0.03</td>
<td>0.22±0.10</td>
<td>1.0±0.12†</td>
<td>0.98±0.21†</td>
</tr>
<tr>
<td>SDF-1</td>
<td>0.07±0.01</td>
<td>0.08±0.02</td>
<td>0.15±0.02†</td>
<td>0.13±0.01†</td>
</tr>
<tr>
<td>MI, 14 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen Iα1</td>
<td>0.07±0.005</td>
<td>0.05±0.001</td>
<td>1.2±0.06†</td>
<td>2.1±0.3†</td>
</tr>
<tr>
<td>Collagen III</td>
<td>0.03±0.01</td>
<td>0.03±0.003</td>
<td>0.37±0.03†</td>
<td>0.68±0.07†</td>
</tr>
<tr>
<td>tTG</td>
<td>0.39±0.06</td>
<td>0.23±0.05</td>
<td>0.88±0.13†</td>
<td>0.76±0.11†</td>
</tr>
</tbody>
</table>
| ND indicates not detectable; MCP-1, monocyte chemoattractant protein; RANTES, regulated-on-activation, normal T-cell–expressed and –secreted chemokine; TNF, tumor necrosis factor; and SDF-1, stromal cell–derived factor 1.

Increased Activity of MMPs in the Absence of Synd1

Inflammatory cells in the infarct are the main source of MMP-2 and -9, proteolytic enzymes involved in cardiac dilatation and dysfunction after MI. Increased inflammation in the absence of Synd1 was related to significantly enhanced levels of pro–MMP-2 and -9 enzyme activity in Synd1 KO compared with WT infarcts at 14 days after MI (n=5 per group; P<0.05; Figure 5A). Immunoblotting confirmed the identity of the proform of MMP-2 and MMP-9, and semiquantitative analysis showed increased expression of pro–MMP-2 and -9 in Synd1 KO compared with WT infarcts (Figure 5B). Baseline pro–MMP-2 or -9 protein and activity levels did not significantly differ between Synd1 WT and KO mice (online Data Supplement, Section III).

MMP-2 inactivates tTG, an enzyme known to stabilize the ECM and protect against cardiac dilatation. The activity level of tTG was significantly decreased in infarcts of Synd1 KO compared with WT mice but did not differ between sham-operated hearts (Figure 4A and 4B, Tables 1 and 2). Sirius red polarization microscopy revealed mainly well-aligned and thick, tightly packed (orange-red) collagen fibers in WT infarcts (Figure 4C). In contrast, loosely assembled (yellow-green) collagen fibers predominated in Synd1 KO infarcts at 14 days (ratio of orange-red to yellow-green birefringent collagen in the infarct area at 14 days; Figure 4C and 4D, Table 2). Ultrastructural analysis confirmed a disorganized matrix with a predominance of smaller and fragmented collagen fibers in Synd1 KO infarcts, in contrast to the well-organized collagen matrix with uniform and sharply delineated collagen fibers in WT infarcts (Figure 4E and 4F).

Thus, increased inflammation and accelerated infarct healing in the absence of Synd1 resulted in increased collagen deposition, but of impaired quality.

**TABLE 2. Histological Analysis and Determination of Collagen in Infarcted Area**

<table>
<thead>
<tr>
<th></th>
<th>Synd1 WT, MI, 7 d (n=8)</th>
<th>Synd1 KO, MI, 7 d (n=9)</th>
<th>Synd1 WT, MI, 14 d (n=10)</th>
<th>Synd1 KO, MI, 14 d (n=11)</th>
<th>Adv R5, MI, 14 d (n=8)</th>
<th>Adv Synd1, MI, 14 d (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size, %</td>
<td>44±1.8</td>
<td>43±1.8</td>
<td>45±2.8</td>
<td>43±2.1</td>
<td>45±3.3</td>
<td>47±3.6</td>
</tr>
<tr>
<td>Residual necrotic area, %</td>
<td>35±2.3</td>
<td>21±2.9*</td>
<td>16±4.1†</td>
<td>1.3±0.7†</td>
<td>6.6±0.8</td>
<td>10.0±0.8†</td>
</tr>
<tr>
<td>Infarct thickness, μm</td>
<td>350±15</td>
<td>280±16*</td>
<td>280±19</td>
<td>205±10†</td>
<td>201±17</td>
<td>258±30*</td>
</tr>
<tr>
<td>Leukocyte infiltration, cells/mm²</td>
<td>624±55</td>
<td>934±61*</td>
<td>360±95†</td>
<td>660±64*</td>
<td>469±38</td>
<td>264±31*</td>
</tr>
<tr>
<td>Capillary growth, vessels/mm²</td>
<td>210±7.3</td>
<td>260±10</td>
<td>220±5.9</td>
<td>240±20*</td>
<td>210±10</td>
<td>210±33</td>
</tr>
<tr>
<td>Coronary growth, vessels/mm²</td>
<td>20±1.6</td>
<td>34±1.9*</td>
<td>41±4.4†</td>
<td>40±4.6</td>
<td>37±6.8</td>
<td>34±3.8</td>
</tr>
<tr>
<td>Myofibroblast, % area</td>
<td>16±1.8</td>
<td>32±2.6*</td>
<td>3.7±1.7†</td>
<td>0.7±0.3†</td>
<td>47±1.2</td>
<td>8.4±2.0</td>
</tr>
<tr>
<td>Collagen deposition, %</td>
<td>22±3.2</td>
<td>34±3.0*</td>
<td>38±2.2†</td>
<td>45±2.0†</td>
<td>36±1.5</td>
<td>29±2.2*</td>
</tr>
<tr>
<td>Ratio O-R thick/Y-G thin collagen fibers</td>
<td>ND</td>
<td>ND</td>
<td>7.5±0.4</td>
<td>1.6±0.6*</td>
<td>65±1.5</td>
<td>15±3.0*</td>
</tr>
<tr>
<td>Collagen μg/mL microplate analysis</td>
<td>1.3±0.08</td>
<td>1.6±0.11*</td>
<td>1.6±0.07</td>
<td>2.0±0.09*</td>
<td>1.5±0.008</td>
<td>0.81±0.13*</td>
</tr>
</tbody>
</table>

Adv indicates adenovirus; ND, not detected; O-R, orange-red; and Y-G, yellow green.

*P<0.05 in Synd1 KO vs WT mice; †P<0.05 in infarcted (MI) vs sham-operated mice.

†P<0.05 at 14 vs 7 days.
Synd1 KO compared with WT mice at 14 days \( (n=6\) per group; \( P<0.05; \) Figure 5C), whereas total protein levels determined by immunoblotting did not significantly differ (Figure 5D). Decreased tTG activity resulted in decreased collagen cross-linking, as suggested by a decrease in immunoreactivity of \( \epsilon \)-lysyl \( \gamma \)-glutaminyl cross-links in infarcts\(^{12} \) (Figure 5E and 5F). Baseline protein or activity levels of tTG did not differ significantly in Synd1 WT compared with KO sham mice (online Data Supplement, Section III).

Thus, increased collagen fragmentation and cardiac dilatation in mice lacking Synd1 were related to increased MMP activity and decreased tTG activity.

**Increased Cardiac Dilatation and Systolic Dysfunction in the Absence of Synd1**

Echocardiographic analysis revealed increased cardiac dilatation and impaired systolic function in Synd1 KO compared with WT infarcted hearts at 14 days (Table 3). Increased cardiac dilatation was accompanied by significantly decreased infarct thickness in Synd1 KO mice (Table 3), indicating increased infarct expansion. Increased cardiac failure after MI in Synd1 KO mice was further confirmed by a significant increase of \( \approx 110\% \) in the ratio of lung to body weight in Synd1 KO compared with a nonsignificant increase of \( \approx 25\% \) in WT mice at 14 days after MI (Table 3). Heart rate did not differ significantly between groups (Table 3).
Synd1 Gene Overexpression Prevents Cardiac Dilatation and Dysfunction

To investigate whether overexpression of Synd1 may prevent increased cardiac inflammation, dilatation, and dysfunction after MI, a replication-deficient adenovirus overexpressing Synd1 was injected into Synd1 WT mice, resulting in an ≈30-fold increase in Synd1 immunoblotting in blood at 7 days after MI (online Data Supplement, Section IV). Overexpression of Synd1 significantly reduced cardiac inflammation in the infarct, improved quality of collagen (Figure 6A through 6F, Table 2), and

TABLE 3. Echocardiographic Analysis

<table>
<thead>
<tr>
<th></th>
<th>Synd1 WT, Sham, 14 d</th>
<th>Synd1 KO, Sham, 14 d</th>
<th>Synd1 WT, Infarct, 14 d</th>
<th>Synd1 KO, Infarct, 14 d</th>
<th>AdV R5 Infarct, 14 d</th>
<th>AdV Synd1, Infarct, 14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=8)</td>
<td>(n=10)</td>
<td>(n=11)</td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>LV/BW ratio, mg/g</td>
<td>3.7±0.2</td>
<td>3.5±0.1</td>
<td>4.4±0.2†</td>
<td>4.2±0.3†</td>
<td>4.5±0.1†</td>
<td>4.3±0.2†</td>
</tr>
<tr>
<td>Lung/BW ratio, mg/g</td>
<td>6.0±0.6</td>
<td>5.9±0.3</td>
<td>8.0±0.8</td>
<td>13.4±1.9†</td>
<td>6.5±0.4</td>
<td>7.8±0.3*</td>
</tr>
<tr>
<td>PW diast, mm</td>
<td>0.73±0.03</td>
<td>0.74±0.03</td>
<td>0.82±0.1</td>
<td>0.84±0.08</td>
<td>0.81±0.05</td>
<td>0.78±0.02</td>
</tr>
<tr>
<td>SW diast, mm</td>
<td>0.82±0.02</td>
<td>0.81±0.03</td>
<td>0.92±0.1</td>
<td>0.91±0.07</td>
<td>0.93±0.04</td>
<td>0.94±0.03†</td>
</tr>
<tr>
<td>LV EDD, mm</td>
<td>4.3±0.1</td>
<td>4.1±0.2</td>
<td>5.5±0.2†</td>
<td>6.6±0.3†</td>
<td>4.9±0.03†</td>
<td>4.1±0.2*</td>
</tr>
<tr>
<td>LV ESD, mm</td>
<td>3.2±0.1</td>
<td>3.0±0.1</td>
<td>4.6±0.3†</td>
<td>5.6±0.4†</td>
<td>3.8±0.1</td>
<td>3.0±0.1*</td>
</tr>
<tr>
<td>FS, %</td>
<td>27±2.1</td>
<td>26±2.4</td>
<td>18±2.6†</td>
<td>10±1.0†</td>
<td>22±0.8†</td>
<td>28±1.2*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>480±31</td>
<td>490±22</td>
<td>450±25</td>
<td>470±29</td>
<td>490±20</td>
<td>500±12</td>
</tr>
</tbody>
</table>

AdV indicates adenovirus; AdV, adenoviral gene overexpression of a control (R5) gene compared with syndecan-1 (Synd1) gene; LV, left ventricular; BW, body weight; PW, noninfarcted posterior wall; diast, diastolic; SW, noninfarcted septal wall; EDD, end-diastolic diameter; ESD, end-systolic diameter; and FS, fractional shortening.

*P<0.05 in Synd1 KO vs WT mice and in AdV Synd1-treated vs control AdV R5-treated WT mice.
†P<0.05 in infarcted vs sham mice.

Figure 5. Increased MMP and decreased tTG cross-linking in Synd1 KO infarcts. A, B, Significantly increased zymographic activity (A) and protein expression (B) of pro–MMP-2 and -9 in Synd1 KO vs WT mice at 14 days after MI. C, D, Significantly decreased activity (C) of tTG but unchanged protein expression (D) in infarcts of Synd1 KO vs WT mice at 14 days.

Figure 6. Synd1 overexpression improves infarct healing. A, B, Hematoxylin and eosin staining revealed a lower degree of removal of necrotic cardiomyocytes in Synd1-treated vs control R5-treated infarcts at 14 days. C, D, Significantly decreased number of CD45-immunoreactive inflammatory cells in Synd1-treated vs control R5-treated infarcts at 14 days. E, F, Increased ratio of well-aligned and thick, tightly packed (orange-red) collagen fibers to loosely assembled (yellow-green) fibers in Synd1-treated infarcts.
prevented cardiac dilatation and dysfunction at 14 days after MI (Table 3).

Thus, overexpression of Synd1 clearly protects against adverse infarct healing, thereby reducing ventricular dilatation and failure after MI.

Discussion

The present study unveils a novel role for Synd1 in protecting against adverse cardiac remodeling and dysfunction after MI. Whereas the absence of Synd1 in mice resulted in accelerated infarct healing with increased cardiac dilatation and dysfunction after MI, adenoviral gene overexpression of Synd1 reduced cardiac inflammation and protected against cardiac dilatation and dysfunction after MI.

Influx of inflammatory cells into the necrotic area is the earliest event in infarct healing. These inflammatory cells degrade the extracellular matrix surrounding the cardiomyocytes, alter the expression and activity of growth factors and chemokines, and thereby clear the way for the influx of other wound-healing cells. Expression of Synd1 is most pronounced in the periinfarct region, spatially and temporally related to inflammation.3.9 Expression of Synd2, 3, and 4 also significantly increases after MI,10 but lack of Synd1 did not result in compensatory changes in Synd2, 3, or 4 after MI, as observed in the present study.

The absence of Synd1 resulted in increased cardiac inflammation and failure, whereas overexpression of Synd1 protected against cardiac dilatation and failure after MI. Increased inflammation in the absence of Synd1 resulted in increased expression of monocyte chemoattractant protein-1 and increased activity of MMP-2 and -9, factors involved in aggravating cardiac dilatation and dysfunction after MI.13–17,24,25 Histological and molecular analyses revealed formation of a less qualitative collagen matrix in the absence of Synd1, which was associated with increased MMP-2 and -9 activity and decreased tTG activity. These findings indicate a central role for Synd1 in regulating infarct inflammation and healing and in preventing cardiac dilatation and dysfunction after MI.

Increased Synd1 may reduce cardiac inflammation after MI by functioning as a barrier against invading inflammatory cells. In our present in vitro study, we observed increased adhesion and transendothelial transmigration of leukocytes lacking Synd1. Previous reports (reviewed elsewhere27) demonstrated that Synd1 decreases leukocyte-endothelial interactions by masking adhesion ligands on endothelial cells and by competing with chemokines that mediate recruitment of inflammatory cells. Of additional interest in this regard are the findings that Synd1 increases adhesion of inflammatory cells to collagens and limits their invasion into collagen gels.28

 Synd1 also may modulate matrix assembly (reviewed previously29,30). The absence of Synd1 resulted in the formation of less qualitative collagen fibers after MI, whereas overexpression of Synd1 improved collagen quality and protected against cardiac dilatation. Increased inflammation in infarcts of mice lacking Synd1 resulted in increased MMP-2 and -9 activity and decreased tTG activity, both contributing to abnormal collagen formation and increased cardiac dilatation and dysfunction after MI.13,15,17 Although a downregulation of MMP-9 expression has previously been linked to increased Synd1 expression in myo-
eloma cells, increased MMP activity in our experiments most likely resulted from increased inflammation, whereas decreased tTG activity may arise from increased degradation by MMP-2.12 A link between increased MMP activity and changes in collagen composition is suggested by different studies,11,14–16 revealing that thick type I collagen fibers are degraded by increased MMPs and replaced by fibrous interstitial deposits of poorly cross-linked collagen. Reduced levels of tTG also contribute to abnormal collagen fibrillogenesis and parallels cardiac dilatation and dysfunction.13,14 tTG mediates the assembly of collagen monomers into fibers, which occurs in close association with the cell surface and involves collagen-binding β1 integrins.26 tTG also stabilizes the extracellular matrix by introducing ε-lysyl γ-glutamyl cross-links into collagens.26

The present study demonstrates the importance and therapeutic potential of Synd1 in ventricular remodeling after MI. Our observations are concordant with previous findings indicating a general role for glycoproteins and matricellular proteins in preventing adverse infarct healing and dysfunction after MI,29 as described for osteopontin30 and thrombospondin-1.1.31 Although we show that a reduction in inflammation by Synd1 is a putative mechanism, the present study cannot definitively answer which processes modulated by Synd1 are pivotal in its protective role.

Our present study reveals a novel role for Synd1 in protecting against infarct healing, cardiac dilatation, and dysfunction after MI. Therefore, Synd1 deserves further investigation as a novel therapeutic tool to reduce cardiac dilatation and failure after MI.

Acknowledgments

We would like to thank R. Geoe and B. Pers for technical assistance.

Sources of Funding

This study was supported by a research grant of the Leuven University, Belgium (OT-0346) to Dr Heymans, a Dr Dekkers grant of the Netherlands Heart Foundation (NHS, 2003T036) to Dr Heymans, a Muenster University Hospital Innovative Medizinische Forschung grant (IMF GO 1 2 04 15) to Dr Götte, and a VIDI grant of the Netherlands Organisation for Scientific Research to Dr Pinto (016.036.346). Dr Pinto is an established investigator of the Netherlands Heart Foundation.

Disclosures

None.

References

Acute myocardial infarction (MI) produces abrupt left ventricular dilatation and global systolic dysfunction. Neurohumoral activation, infarct expansion, and early ventricular remodeling aim to restore stroke volume but are the cause of progressive cardiac dilatation and heart failure. Angiotensin-converting enzyme inhibitors and β-blockers attenuate the continuous neurohumoral activation and thereby prevent late cardiac hypertrophy and dilatation after MI. Hence, therapeutic tools that strengthen the infarct scar, prevent infarct expansion, and hinder ventricular dilatation within the first hours to days after MI are missing. The present study proposes syndecan-1 as a novel therapy to reinforce the infarct scar and to prevent infarct expansion immediately after MI. Gene inactivation of syndecan-1 in mice resulted in impaired collagen quality and impaired collagen cross-linking during early LV rupture and late remodeling after experimental myocardial infarction. Am J Physiol Heart Circ Physiol. 2003;285: H1229–H1235.

Clinical Perspective

Exaggerated left ventricular dilation and reduced collagen deposition following myocardial infarction indicates a role in cardiac remodeling. Am J Pathol. 2001;159:2049–2059.

Exaggerated left ventricular dilation and reduced collagen deposition following myocardial infarction indicates a role in cardiac remodeling. Am J Pathol. 2001;159:2049–2059.
Increased Expression of Syndecan-1 Protects Against Cardiac Dilatation and Dysfunction After Myocardial Infarction

Davy Vanhoutte, Mark W.M. Schellings, Martin Götte, Melissa Swinnen, Veronica Herias, Martin K. Wild, Dietmar Vestweber, Emmanuel Chorianopoulos, Víctor Cortés, Attilio Rigotti, Mary-Ann Stepp, Frans Van de Werf, Peter Carmeliet, Yígal M. Pinto and Stephane Heymans

*Circulation* 2007;115:475-482; originally published online January 22, 2007;
doi: 10.1161/CIRCULATIONAHA.106.644609

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/4/475

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2007/01/26/CIRCULATIONAHA.106.644609.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org/subscriptions/