S100B Expression Modulates Left Ventricular Remodeling After Myocardial Infarction in Mice

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Background—S100B, a 20-kDa, Ca\(^2+\)-binding dimer, is a putative intrinsic negative regulator of myocardial hypertrophy expressed after myocardial infarction. S100B-overexpressing transgenic (TG) and S100B-knockout (KO) mice have been generated to assess the consequences of S100B expression and altered hypertrophy after infarction.

Methods and Results—We compared 21 wild-type (WT), 20 TG, and 24 KO mice over 35 days after experimental myocardial infarction with sham-operated controls (n=56). Of those, 4 WT-infarcted mice, 7 TG-infarcted mice, and 1 KO-infarcted mouse and no sham-operated mice died during the observation period. Among survivors, echocardiography, hemodynamic studies, and postmortem examination indicated that the WT and KO groups of infarcted mice mounted a hypertrophic response that was augmented in KO mice. The S100B-overexpressing TG group did not develop hypertrophy but demonstrated increased apoptosis. The postinfarct end-diastolic pressure was lower in KO mice than in WT mice, in accordance with other structural, hemodynamic, and functional parameters, which suggests that abrogation of S100B expression augmented hypertrophy, decreased apoptosis, and was beneficial to preservation of cardiac function within this time frame.

Conclusions—S100B regulates the hypertrophic response and remodeling in the early postinfarct period and represents a potential novel therapeutic target. (Circulation. 2005;111:598-606.)

Key Words: hypertrophy • proteins • myocardial infarction • apoptosis • remodeling

The structural and functional consequences of myocardial infarction (MI) are related to alterations in left ventricular (LV) geometry called post-MI ventricular remodeling. These changes involve myocyte necrosis and apoptosis, scar formation, ventricular dilation, and hypertrophy of noninfarcted myocardium. Hypertrophy of noninfarcted muscle is regulated by a balance of positive effectors such as \(\alpha_1\)-adrenergic agonists, angiotensin II, and peptide growth factors and negative effectors such as myocyte-enriched calcineurin-interacting protein, mitogen-activated protein kinase-phosphatase-1, and potentially S100B. S100B, a 20-kDa EF-hand Ca\(^2+\)-binding dimer, is a member of a multigenic family of calcium-binding proteins that exhibit tissue-specific expression and has been implicated in the regulation of a number of intracellular activities by its interaction with effector proteins. S100B is expressed in cell types that constitute the nervous system (eg, astrocytes and neuronal subpopulations) and other cell lineages (eg, skeletal muscle cells). S100B can also be released from cells and interact in a paracrine (eg, microglia) or autocrine (eg, astrocytes) manner with trophic or apoptotic consequences depending on the concentration attained. We have identified S100B as a putative intrinsic negative-feedback regulator of the hypertrophic response induced in the myocardium after MI in human subjects and experimental rodent models and have shown it to be capable of inhibiting the \(\alpha_1\)-adrenergic–induced hypertrophy in cultured neonatal rat cardiac myocytes and transgenic mice. In addition to induction of S100B, the post-MI hypertrophic response is accompanied by induction of a program of fetal gene reexpression that encompasses \(\beta\)-myosin heavy chain (\(\beta\)-MHC), the skeletal isoform of \(\alpha\)-actin (skACT), and atrial natriuretic factor (ANF). Although we have shown that S100B inhibits \(\beta\)-protein kinase C signaling in cultured cardiac myocytes, the exact molecular mechanisms through which S100B modulates hypertrophy remain to be defined.

Although teleologically, the myocardial hypertrophy after MI appears to be a manifestation of an early adaptive compensatory response to mitigate the consequences of loss of cardiac muscle, this concept has not been fully tested in animal models, and both positive and negative effectors of the hypertrophic response are potential therapeutic targets to modify this response. To gain further insight into the action of S100B in post-MI myocardial hypertrophy and ventricular remodeling, we used S100B transgenic (TG) mice that overexpress S100B and S100B-knockout (KO) mice that
do not express S100B. We used these mouse models to examine the effects of a potentially attenuated (TG mice) or exaggerated (KO mice) hypertrophic response on survival and LV architecture after experimental MI. Our results indicate that KO mice exhibit greater hypertrophy, less LV dilation, less apoptosis, and improved hemodynamics in the early post-MI period.

**Methods**

**Mice**

S100B TG mice containing multiple copies (>8) of the human S100B gene under the control of its own promoter and S100B-KO mice with a null mutation of S100B were derived on a CD1 background as described previously.2,13 TG, KO, and control wild-type (WT) CD1 mice (Charles River, St. Constant, Quebec, Canada) were housed in microisolators. All animal experiments conformed to protocols approved by St. Michael’s Hospital and University Health Network Animal Care Committees.

**Left Coronary Artery Ligation**

Left anterior descending (LAD) coronary artery ligation was performed as described previously.14 In brief, 8-week-old mice were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally. A left thoracotomy was performed, and a 7-0 silk suture was placed through the myocardium into the anterolateral LV wall. LAD-ligated mice were maintained for 35 days and were compared with sham-operated (nonligated) mice.

**Monitoring of Systolic Pressure and Heart Rate**

Because the anesthetics ketamine and xylazine induce a marked negative chronotropic effect,15 on day 35 after coronary artery ligation, LV systolic blood pressure (LVSP) and heart rate (HR) were measured with an indirect mouse tail blood pressure system (Harvard Apparatus) according to the instructions supplied by the manufacturer.

**Echocardiographic Assessment of Cardiac Hypertrophy and Function**

Transthoracic echocardiography, with a 12-MHz probe (Hewlett Packard),3 was performed with mice anesthetized with 1% isofluorane (Halocarbon) using a vaporizer and 0.2 L/min oxygen. LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters, end-diastolic interventricular septal (SWT), and anterolateral (LWT) wall thickness, and LV percent fractional shortening (FS) were measured from M-mode tracings (short-axis view) at the level of the papillary muscles and recorded as the consensus of 2 blinded observers (J.N.T. and T.G.P.) with interobserver variability <10%. FS was calculated with the following equation: 

\[
FS(\%) = \left( \frac{LVEDD - LVESD}{LVEDD} \right) \times 100.
\]

**In Situ Hemodynamics**

For in situ measurements, the animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally, and LV pressures were measured by fluid-filled (heparinized saline [100 U/mL]) catheters as previously described6 or by a Millar microtip catheter transducer. Mean arterial pressure (MAP) was calculated with the following equation: MAP (mm Hg) = 1/3(SP – DP) + DP, where SP is systolic pressure and DP is diastolic pressure. There were no significant differences in the hemodynamic measurements obtained by fluid-filled catheters compared with the Millar microtip catheter in any experimental group.

**Harvesting of Cardiac Tissue**

On day 35 after LAD ligation, mice were euthanized to harvest cardiac tissue for measurement of LV and right ventricular weights, RNA and protein isolation, and fixation.7 Infarct size was determined as described previously.16 Infarct size (in percent) was calculated as total infarct circumference divided by total LV circumference times 100.

**Ribonuclease Protection Assay**

RNA was isolated from tissues by a 1-step acid guanidinium phenol method.17 RNase protection assays to determine steady-state levels of S100B mRNA, ANF mRNA, β-MHC mRNA, and GAPDH mRNA were performed as described previously.5

**Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction**

RNA was extracted as above and analyzed with thermoscript 1-step quantitative reverse transcription–polymerase chain reaction (RT-PCR) with the platinum Taq kit to synthesize cDNA and subsequent real-time quantitative RT-PCR (Applied Biosystems). The gene-specific sequences of oligonucleotide primers (Qiagen) were based on DNA sequences in the National Center for Biotechnology database unless otherwise stated and were as follows: human S100B, TGGACAAATGTAGGAGACGG (forward), ATTAGTCAACACCCGCTTG (reverse); mouse S100B, 18 GCGTACACCATGTC-CCCTGTAG (forward), CTGGGCCATCTCTCTCTGT (reverse); ANF, CCTGGGCCACCTCTCTTAATA (forward), AGGCCCTCGATTTTCCA (reverse); β-MHC, GTGCCCAAAGGCCTG-GAATGAG (forward), GCAAAGGCCTGAGGCTGTA (reverse); skACT, AGGAAGGACCTGTAGCCCA (forward), GTACATG- GTAGTGGCCCTCTGA (reverse); α-MHC, CTCGTCGAGAGGTTATATTCCCTG (forward), GGAAGATGTAGCCGGCCTCACAA (reverse); cardiac sarcolemic reticulum Ca2+-ATPase 2α (SERCA 2α), CGGTTCCTACAGGGCGAG (forward), ACCAGATTGCCACCTAACTAG (reverse); ryanodine receptor,20 GACGGCGAAGGCACTACCTCGCC (forward), CTCGCAAGAAAAGCTGACATG (reverse); GAPDH, GTGCACTGGCCAGCTCCTGC (forward), GGACAGTTACAGGCGATG (reverse). The gene-specific oligonucleotide primers were added to a mixture that contained a 1× concentration of SYBR green PCR master mix, 0.25 U/μL multiscrbe reverse transcriptase, 0.4 U/μL RNase inhibitor, and 10 ng of total tissue RNA in a 50-μL mixture according to the manufacturer’s protocol (Applied Biosystems). The relative quantification of gene expression by real-time RT-PCR in a sample was determined by comparing the target-amplified product against GAPDH (internal standard) within the same sample. GAPDH mRNA expression was not significantly different among the various groups. Gene expression in the MI groups was compared with their respective sham group, which was set at 1.

**Western Blotting**

Western blotting was performed on aliquots of tissue extracts that contained 500 μg of total protein or bovine brain S100B (Calbiochem; 5 to 50 ng) as described previously.5

**Cardiac Myocyte Cultures**

Neonatal cardiac myocytes were isolated from the ventricles of 2-day-old WT, TG, and KO mice as described previously.4,5 Cell protein was determined after 48-hour treatment with either vehicle (ascorbic acid 100 μM) or 20 μM norepinephrine (NE) and was quantified by continuous labeling with [14C]phenylalanine as described previously.4 There were at least 3 cell culture dishes in each group. There was no change in the number of cells after treatment of cultures with any agent.

**Measurement of Apoptosis in LV Transverse Sections**

LV transverse sections (4 μm thick) were subjected to the TUNEL (terminal dUTP nick end-labeling) method with the Cardiotoacs In Situ Apoptosis Detection Kit according to the manufacturer’s instructions (R&D Systems). To identify cells or bodies of cardiac origin, the sections were double stained with MF-20, a monoclonal anti-myosin antibody (working dilution 1:50; Developmental Studies
Hybridoma Bank, University of Iowa). A goat anti-mouse TRITC conjugate (Sigma Chemical Co; working dilution 1:200) was used as secondary antibody for LV transverse sections. Thus, for each heart, the number of TUNEL-positive cells (blue nuclei) was scored per total number of myocytes. From each section, 17 light microscopic fields (×400) selected at random that bordered each side of the infarct were used to count the number of cells positively labeled for nuclear DNA fragmentation. The average number of cells in fields selected from regions that bordered infarcts was 98 (range 88 to 108). In addition, 10 fields were selected at random from the infarct and remote myocardial regions. No TUNEL-positive cells were identified in these regions. Sections from the LV free wall from WT sham, TG sham, and KO sham mice were examined in an identical fashion. The average number of cells in these fields was 107 (range 95 to 119). Values are presented as mean ± SEM percent TUNEL-positive myocytes per total myocytes per field (×400), with 6 to 8 sections per group.

Assessment of Apoptosis by DNA Laddering
The methodology used for the assessment of apoptosis by DNA laddering in LV tissue has been described previously.21–23

Figure 1. Comparison of survival of mice after coronary artery ligation (LAD ligation). *P<0.05 vs KO-LAD ligation.

Figure 2. Mice were euthanized 35 days after sham or coronary artery ligation (LAD Ligation). Photomicrograph depicting perfusion-fixed midlevel left and right ventricular transverse slices.

Figure 3. Mice were euthanized 35 days after coronary artery ligation (LAD Ligation). Steady state levels of ANF, β-MHC, GAPDH (A), human S100B, and endogenous mouse S100B mRNAs (B) were determined by RNase protection. Protected fragments specific for human S100B (210 bp), mouse S100B (135 bp), ANF (600 bp), β-MHC (218 bp), and GAPDH (355 bp) mRNAs are shown in composite figures depicting results of representative experiments using RNA from human brain, mouse brain, and fetal and adult mice hearts. Western analysis was performed on heart extracts. Position of migration of S100B is shown in representative blot (C). Purified bovine S100B protein was used as quantitative control.

Analysis of Apoptosis in Neonatal Cultured Mouse Myocytes
Neonatal CD1, KO, and TG mouse cardiac myocytes were analyzed for apoptosis by the TUNEL method or after visualization of nuclear DNA fragmentation.
morbidity in situ was defined as the relative expression of S100B dimer, the concentration of S100B in total ven- tricular tissue of TG and WT mice after infarction is 60 and 600 by light or fluorescent microscopy and analyzed for apoptotic characteristics. Ten fields were selected at random per culture dish for quantification. The average number of cells in these fields was 80 (range 70 to 90). The apoptotic index (percentage of apoptotic nuclei) was calculated as (myocyte apoptotic nuclei/total myocyte nuclei)×100. Sample identities were concealed during scoring, and at least 6 culture dishes were scored per group.

**Statistical Analysis**
Treated/control ratios were tested for deviation from unity by calculation of confidence limits. Mean values were compared by 1-way ANOVA followed by a Dunnett test when appropriate and by Student’s t test where appropriate. Only probability values of *P*<0.05 were accepted as statistically significant.

**Results**

**MI and Survival**
A total of 136 mice were studied, 80 that underwent LAD ligation and 56 that were sham-operated. Of those undergoing LAD ligation, 15 (5 WT, 6 TG, and 4 KO mice) died within 24 hours and were excluded from the study. All sham-operated mice survived surgery and to the end of the observation period. Among LAD-ligated mice, there was 1 death in KO mice (1/24), 4 deaths in WT mice (4/21), and 7 deaths in TG mice (7/20). Death in TG mice occurred due to rupture of the LV. There was a significant difference in survival between KO and TG mice (*P*=0.01; Figure 1). No sham-operated mice but all LAD-ligated mice developed LV transmural infarction and subsequent scar formation (Figure 2). Infarct sizes for the groups of mice studied were 41±4% (WT), 43±4% (TG), and 38±3% (KO), with extensive apical involvement, which was not significantly different from each other.

**Induction of S100B and Fetal Gene Program**
Hearts of euthanized mice were examined for evidence of post-MI gene expression with RNase protection and quantified by real-time RT-PCR (Figures 3A and 3B; Table 1).

**TABLE 1. LV Gene Expression After Coronary Artery Ligation**

<table>
<thead>
<tr>
<th></th>
<th>α-MHC</th>
<th>β-MHC</th>
<th>skACT</th>
<th>ANF</th>
<th>Ryanodine Receptor</th>
<th>SERCA 2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-SHAM</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>WT-LAD Lig.</td>
<td>0.71±0.11</td>
<td>1.89±0.21*</td>
<td>1.56±0.31</td>
<td>1.79±0.23*</td>
<td>0.44±0.13*</td>
<td>0.59±0.11*</td>
</tr>
<tr>
<td>TG-SHAM</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TG-LAD Lig.</td>
<td>0.82±0.11</td>
<td>0.78±0.09</td>
<td>0.71±0.09</td>
<td>1.01±0.36</td>
<td>0.73±0.11</td>
<td>1.33±0.30</td>
</tr>
<tr>
<td>KO-SHAM</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>KO-LAD Lig.</td>
<td>0.38±0.05*</td>
<td>2.47±0.49*</td>
<td>2.96±0.27†</td>
<td>6.07±0.54†</td>
<td>0.31±0.03*</td>
<td>0.55±0.12*</td>
</tr>
</tbody>
</table>

LAD Lig. indicates coronary artery ligation. All values are mean±SEM of ratio of respective gene (relative to GAPDH expression) after LAD ligation to sham treatment (set at 1).

S100B was not detected in sham-operated or KO mice by RNase protection. After MI, both the human S100B transgene and the mouse endogenous S100B gene were induced in TG mice, and the mouse endogenous S100B gene was induced in WT mice (Figure 3B; 10.65±0.89-fold, 1.79±0.23-fold, and 2.11±0.15-old, respectively, by real-time RT-PCR, *n*=6). There was no induction of the hypertrophic fetal genes ANF, β-MHC, and skACT in sham-operated or TG LAD-ligated mice 35 days after injury (Figure 3A; Table 1). Fetal gene expression was induced in WT and, to a greater magnitude, KO mice (Figure 3A; Table 1). α-MHC and the calcium binding proteins, SERCA 2a and the ryanodine receptor, were downregulated in WT and KO mice (Table 1). By quantitative Western blot analysis, the band immunoreactive with S100B from the LV of TG-ligated mice was of greater intensity than that observed in WT-ligated mice, corresponding to ≈10 and 1 ng of S100B protein per 500 μg of total myocardial protein. Assuming a molecular weight of 20 kDa for S100B dimer, the concentration of S100B in total ventricular tissue of TG and WT mice after infarction is ≈60 and 6 nmol/L, respectively (Figure 3C).

**LV Weight and Myocyte Protein**
Morphometric analysis confirmed that the hypertrophy of the noninfarcted muscle was blunted in TG mice overexpressing S100B and exaggerated in KO mice that did not express S100B (Figure 2). All LAD-ligated and sham-operated mice had similar total body weights (data not shown); however, the LV weight/body weight ratios were significantly increased in LAD-ligated WT and KO mice, but not in TG mice, compared with their respective sham-operated controls (Figure 4A). Moreover, the increase in KO mice was significantly higher than in WT mice (Figure 4A). LAD ligation had no significant effect on the right ventricle weight/body weight or lung weight in any group of mice (data not shown). To confirm a direct action of S100B on cardiac myocytes, primary myocyte cultures were established from hearts of neonatal, WT, TG, and KO mice. The α1-adrenergic agent NE did not increase myocyte protein content in TG myocytes but increased cell protein content 1.3-fold in WT myocytes and provoked a significantly greater 1.7-fold increase in KO myocytes (Figure 4B).

**Echocardiographic Measurements**
At day 35, echocardiographic parameters of sham-operated WT, TG, and KO mice were not significantly different. SWT
increased significantly in infarcted WT and KO mice but not TG mice over their respective sham-operated controls, and this increase was significantly higher in KO mice than in WT mice (Table 2). All groups exhibited significant decreases in infarcted LWT and increases in dilation of the LV chamber, on the basis of increased LVEDD and LVESD compared with respective sham-operated mice. However, there was less thinning of the infarcted LV wall and less dilation of the LV chamber in KO mice than in WT or TG mice (Table 2). Thus, in the absence of S100B, LV architecture could be interpreted as more favorable for post-MI recovery. Conversely, overexpression of S100B in TG mice promoted increased dilatation of the LV chamber.

**LV Function After LAD Ligation**

We also examined post-MI changes in parameters related to cardiac function in vivo 35 days after surgery before euthanasia. In all groups of mice, there was a decrease in FS, LVSP, and MAP and an increase in LVEDP compared with sham-operated controls (Tables 2 and 3). The increase in LVEDP and the fall in FS were significantly attenuated in KO mice compared with WT mice (Tables 2 and 3).

**LV Apoptosis After LAD Ligation**

We also examined the hearts of euthanized mice for evidence of apoptosis at 7, 14, and 35 days after experimental MI. DNA samples from peri-infarct cardiac tissue displayed the laddering pattern of fragmented DNA characteristic of apoptosis beginning at 7 days after LAD ligation in TG mice and 14 days after LAD ligation in WT mice. Thirty-five days after LAD ligation, all groups displayed DNA fragmentation that was absent in corresponding sham-operated mice (Figure 5A). We also determined the relative number of apoptotic cells in the peri-infarct region by TUNEL staining in LV sections of WT, TG, and KO sham-operated mice and 35 days after LAD ligation (Figure 5B). A significantly greater number of apoptotic cells were detected in all LAD-ligated mice than in their respective sham-operated controls (Figure 5B). The percent of apoptotic over viable cardiac myocytes was highest in TG mice (0.22±0.03%), intermediate in WT mice (0.13±0.03%), and lowest in KO mice (0.06±0.03%), with a significant difference between KO mice and TG mice (Figure 5B).

**Cardiac Myocyte Apoptosis and S100B**

To address the question of whether exogenous S100B can induce cardiac myocyte apoptosis directly, primary cultures were established from hearts of WT, TG, and KO neonatal mice. Treatment of WT myocyte cultures with increasing doses of S100B caused DNA fragmentation as visualized by TUNEL (Figure 6A). Myocyte apoptosis was confirmed by colabeling with the myocyte-specific marker MF-20 and visualization with horseradish peroxidase staining (Figure 6B). The small nonmyocyte population did not undergo S100B-induced apoptosis (Figure 6B). Myocyte apoptosis–associated nuclear morphology, identified by the fluorescent DNA-binding dye Hoechst 33342 and colabeling by MF-20, was performed as a second independent assessment of apoptosis (Figure 6C). Apoptotic cardiac myocytes were counted and expressed as a percentage of total cardiac myocytes. In this quantitative assay, a significant increase in myocyte apoptosis was first observed at 100 nmol/L S100B relative to vehicle-treated myocytes regardless of the methodology used to determine apoptosis (Figure 6C). The known apoptotic stimuli TNF-α and H2O2 also induced cardiomyocyte apoptosis to a degree similar to 10 μmol/L S100B (Figure 6C). The genomic S100B status of cardiomyocytes did not affect their response to extracellular S100B and other apoptotic stimuli,
Table 2. LV Dimensions as Assessed by Echocardiography After Coronary Artery Ligation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SWT, mm</th>
<th>LWT, mm</th>
<th>LVEDD, mm</th>
<th>LVESD, mm</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-Sham</td>
<td>19</td>
<td>0.73±0.01</td>
<td>0.70±0.02</td>
<td>3.62±0.06</td>
<td>2.12±0.11</td>
<td>43.5±1.2</td>
</tr>
<tr>
<td>WT-LAD Lig.</td>
<td>17</td>
<td>0.87±0.02*</td>
<td>0.53±0.02*</td>
<td>4.61±0.32*</td>
<td>4.99±0.30*</td>
<td>24.4±2.3*</td>
</tr>
<tr>
<td>TG-Sham</td>
<td>19</td>
<td>0.76±0.03</td>
<td>0.70±0.01</td>
<td>3.82±0.12</td>
<td>2.36±0.06</td>
<td>37.9±2.3</td>
</tr>
<tr>
<td>TG-LAD Lig.</td>
<td>13</td>
<td>0.75±0.03*</td>
<td>0.48±0.03*</td>
<td>7.81±0.25*</td>
<td>5.55±0.23*</td>
<td>29.2±2.1*</td>
</tr>
<tr>
<td>KO-Sham</td>
<td>19</td>
<td>0.75±0.02</td>
<td>0.74±0.02</td>
<td>4.05±0.18</td>
<td>2.35±0.19</td>
<td>42.5±3.2</td>
</tr>
<tr>
<td>KO-LAD Lig.</td>
<td>23</td>
<td>1.00±0.02†</td>
<td>0.64±0.02†*</td>
<td>5.13±0.13†*</td>
<td>3.28±0.17†*</td>
<td>35.4±4.7†*</td>
</tr>
</tbody>
</table>

LAD Lig. indicates coronary artery ligation. All values are mean±SEM. 
*P<0.05 vs respective sham; †P<0.05 vs WT-LAD.

Discussion

The change in geometry after MI is a progressive process that involves LV chamber dilation, infarcted wall thinning, myocyte apoptosis, and compensatory thickening in the noninfarcted region.1,14,24 In the present study, we used TG and KO mice to assess the role of the calcium-binding protein S100B, with its concomitant effects on hypertrophy, in the early (35 day) period after experimental myocardial infarction provoked by LAD ligation. This approach was based on our previous observations suggesting S100B could act as an intrinsic negative modulator of the hypertrophic response. S100B is induced in the peri-infarct region in human and rat heart, inhibits the activation of fetal genes by α1-adrenergic agonists in cultured myocytes, and limits adrenergic-induced hypertrophy in vivo.4,5

In the present study, induction of an ≈10-fold higher level of S100B protein in TG mice above that seen in WT animals (Figure 2C) inhibited post-MI hypertrophy as assessed by LV/body weight ratio and SWT (Figures 2 and 4; Table 2). By contrast, KO mice demonstrated augmented hypertrophy. Confirming an effect on the full spectrum of the hypertrophic phenotype, upregulation of the fetal genes ANF, β-MHC, and skACT, seen in WT mice, was attenuated in TG and enhanced in KO mice. The above observations support a role for S100B in limiting the hypertrophic response after infarction and confirmed the utility of S100B TG and KO mice as models for altering this response.

In all 3 groups of mice studied, there was thinning of the infarcted LV wall (LWT) and an increase in the LV cavity based on measurements of LVEDD and LVESD relative to sham-operated controls (Table 2). This indicates that other components of postinfarct remodeling, such as “slippage” of muscle fibers in the peri-infarct region and apoptosis, occur even in the context of modifying the hypertrophic response. Indeed, in the context of increased hypertrophy, as is the case in KO mice, there was significantly more mural thinning of the infarct and noninfarct zones and dilatation after MI than in WT mice. This suggested that the enhanced hypertrophic response associated with a block to induction of S100B conferred changes in LV architecture that could be interpreted as more favorable to averting the sequelae in the immediate post-MI period such as LV rupture or early progression to pump failure. The survival data on the 3 groups of mice support this concept, with a significant difference in survival between KO and TG mice.

Consistent with the size of experimental infarction, we found evidence for impaired cardiac function in WT, TG, and KO mice at 35 days after experimental MI, including decreases in FS, LVSP, and MAP and increases in LVEDP, but no overt heart failure. In KO mice, FS was preserved, and notably, the increase in LVEDP was significantly lower than in WT or TG mice. Thus, paradoxically, increased hypertrophy in KO mice, in the setting of less ventricular dilation, was not at the expense of augmented filling pressures and impairment of diastolic filling. Attenuation of LVEDP occurred even with downregulation of SERCA 2a and ryanodine receptor expression in KO mice (Table 1). The impact on preservation of systolic function and ventricular dimensions...
may predominate over the potential adverse effects of the hypertrophic phenotype on filling in the early postinfarct period. These findings are in agreement with those of a previous study in a rat model showing that induction of additional myocardial hypertrophy with an inhibitor of long-chain fatty oxidation reduced LV dilation and preserved function 13 days after MI.25,26

Global changes in cardiac mass and wall thickness after infarction represent the sum of both myocyte hypertrophy and loss or apoptosis. Overlapping pathways can trigger both hypertrophy and apoptosis, and over a chronic course, they can lead to dilated cardiomyopathy and heart failure.27 The postinfarct hypertrophic response in the myocardium is initiated by multiple trophic signals that include the state of local and systemic sympathetic hyperactivity through $\alpha_1$-adrenergic stimulation.28 We have previously provided experimental evidence that the same $\alpha_1$-adrenergic pathway that initiates and sustains the hypertrophic response in cardiac myocytes by activating protein kinase C and is subject to negative modulation by S100B also induces S100B.4,10 Although the exact mechanisms by which S100B modulates myocyte hypertrophy are unknown, this protein is capable of binding directly to multiple intracellular targets, and we and others have provided evidence that intracellular S100B is capable of inhibiting the phosphorylation of kinase substrates.4,6,7 Extracellular S100B inhibits myogenic differentiation of rat L6 myoblasts and the expression of the myogenic differentiation markers, including myosin heavy chain, via inactivation of p38 kinase.29 In the brain, S100B is released by astrocytes and at nanomolar concentrations stimulates neurite outgrowth and survival, whereas at micromolar concentrations, it causes astrocyte and neuronal apoptosis, in part through interaction with the receptor for advanced glycation end products (RAGE).21,30 Analogously, we demonstrate that in neonatal mouse cultures, exogenously administered S100B induces apoptosis in a dose-dependent manner beginning at 100 nmol/L, a local or regional concentration that may be achieved in the peri-infarct myocardium, at least in TG mice (Figure 2C). Although there may be differences in susceptibility to apoptosis between neonatal and adult myocytes, we documented the presence of apoptosis in the peri-infarct region at 35 days after LAD ligation, but not in the heart of sham-operated controls, and confirmed previous observations that apoptosis in peri-infarct myocardium increases with time.
after infarction. Moreover, we found significantly fewer apoptotic cells in the heart of KO mice than in TG mice at day 35 after infarction (Figure 5B). Furthermore, peri-infarct myocyte apoptosis appears earlier at day 7 in TG mice, which coincides with our previously demonstrated timing of induction of S100B at day 7, peaking at day 28.4 S100B may play a role in the regulation of apoptosis in post-MI myocardium by an extracellular mechanism after cellular release from damaged myocytes and interaction with RAGE, resulting in cytochrome c release and the activation of the proapoptotic caspase cascade, as has been reported in N18 neuroblastoma cells in response to micromolar concentrations.30 Relevant to the postinfarct setting, S100B effects overlap and interact with oxidative stress–activated pathways in this cell lineage.30 Alternatively, S100B may induce apoptosis in a RAGE-independent manner by interacting with an unidentified receptor, as has been reported in myoblast cell lines via stimulation of reactive oxygen species production and inhibition of the prosurvival kinase ERK1/2.32 Finally, the present studies do not exclude that the intracellular effects of S100B may themselves modulate the apoptotic response of postinfarct myocytes. In total, S100B expression after infarction may modulate global remodeling by distinct intracellular and extracellular mechanisms that regulate both myocyte growth and apoptosis.

The effects of S100B on myocyte growth and function stand in contrast to that of S100A1, the most abundant S100 protein expressed in cardiac muscle under basal conditions.6 S100A1 exhibits increased expression in compensated hypertrophy and decreased expression in human cardiomyopathy, and we have previously demonstrated downregulation of this protein after experimental MI.33–35 S100A1 KO and TG mice demonstrate impaired and augmented cardiac contractile responses to stress, respectively.35–37 S100A1 appears to modulate sarcoplasmic reticulum calcium handling and myofibrillar responsiveness.38 We have demonstrated that unlike S100B, S100A1 plays a role in maintenance of differentiated gene expression.23 Like our proposed mechanism for S100B release, S100A1 is released into the extracellular space in the setting of myocardial injury, but unlike S100B, extracellular S100A1 inhibits apoptosis via activation of ERK 1/2.39 Thus, S100 proteins may differentially regulate myocardial structure and function. Given the capacity of S100A1 and S100B to heterodimerize, phenotypic consequences may depend on the availability and stoichiometry of S100A1 and S100B homodimers and heterodimers.

The present study is limited to the impact of S100B expression on the hypertrophic and apoptotic responses in the early period of healing after MI. Our current model does not reflect decompensated LV dysfunction, and the ultimate impact of S100B on the development of congestive heart failure will require a more long-term study that does not focus on the early hypertrophic response. Furthermore, the regulation of S100B expression in the present transgenic model is...
dependent on the gene’s own tissue-restricted promoter, which constrains the ability to experimentally regulate the timing and magnitude of expression. Nevertheless, the present results add to an emerging understanding of the cardiac effects of members of the S100 protein family and specifically demonstrate that S100B expression modulates hypertrophy, apoptosis, and remodeling after infarction. Because absence of S100B in KO mice was associated with structural, functional, and survival advantage, S100B could be a novel therapeutic target in post-MI management.

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References

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