

MCIP1 Overexpression Suppresses Left Ventricular Remodeling and Sustains Cardiac Function After Myocardial Infarction

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Abstract—Pathological remodeling of the left ventricle (LV) after myocardial infarction (MI) is a major cause of heart failure. Although cardiac hypertrophy after increased loading conditions has been recognized as a clinical risk factor for human heart failure, it is unknown whether post-MI hypertrophic remodeling of the myocardium is beneficial for cardiac function over time, nor which regulatory pathways play a crucial role in this process. To address these questions, transgenic (TG) mice engineered to overexpress modulatory calcineurin-interacting protein-1 (MCIP1) in the myocardium were used to achieve cardiac-specific inhibition of calcineurin activation. MCIP1-TG mice and their wild-type (WT) littermates, were subjected to MI and analyzed 4 weeks later. At 4 weeks after MI, calcineurin was activated in the LV of WT mice, which was significantly reduced in MCIP1-TG mice. WT mice displayed a 78% increase in LV mass after MI, which was reduced by 38% in MCIP1-TG mice. Echocardiography indicated marked LV dilation and loss of systolic function in WT-MI mice, whereas TG-MI mice displayed a remarkable preservation of LV geometry and contractility, a pronounced reduction in myofiber hypertrophy, collagen deposition, and β -MHC expression compared with WT-MI mice. Together, these results reveal a protective role for MCIP1 in the post-MI heart and suggest that calcineurin is a crucial regulator of postinfarction-induced pathological LV remodeling. The improvement in functional, structural, and molecular abnormalities in MCIP1-TG mice challenges the adaptive value of post-MI hypertrophy of the remote myocardium. The full text of this article is available online at <http://circres.ahajournals.org>. (*Circ Res.* 2004;94:e18-e26.)

Key Words: myocardial infarction ■ heart failure ■ calcineurin ■ signaling ■ hypertrophy

Despite significant progress in the prevention and treatment of cardiovascular disease in the United States in the past two decades, statistics indicate that the incidence and prevalence of heart failure have been increasing steadily, especially in the elderly. Chronic heart failure affects 4.8 million Americans and is the leading cause of hospitalization for people aged 65 years and over. Despite improved medical treatment and intense investigation, heart failure is a leading cause of morbidity and mortality in industrial countries.^{1,2}

A fundamental shift in the underlying etiology of heart failure is becoming evident, in which the most common cause of chronic heart failure is no longer hypertension or valvular disease, but rather coronary artery disease (CAD) and long-term survival after myocardial infarction (MI).¹ Unfortunately, the prognosis of patients with heart failure and CAD

is considerably worse compared with heart failure without CAD.¹ MI induces scar formation and global changes in surviving myocardium, designated post-MI ventricular remodeling. This process consists of an initial wall thinning of the infarcted area, ventricular chamber dilation, side-to-side slippage, and eccentric myocyte hypertrophy of the individual myofibers in the noninfarcted (remote) portion of the myocardium.³ In spite of clinical evidence that the postinfarcted heart often progressively dilates and displays accelerated deterioration of left ventricular function, the early hypertrophic remodeling of the viable portion of the left ventricle (LV) after ischemic damage is considered an adaptive response to compensate for the acute loss of functional myocardium and preserve cardiac performance.⁴

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Sustained cardiac hypertrophy has been recognized as the single most important risk factor for heart failure development, at least in conditions with increased load such as chronic hypertension or valvular disease, and a powerful predictor for cardiovascular morbidity and mortality.⁵ One signaling pathway that links extracellular stimuli to a hypertrophic transcriptional response of the myocyte uses the Ca^{2+} calmodulin-dependent phosphatase calcineurin and its downstream transcriptional effector nuclear factor of activated T cells (NFAT).^{6,7} Pressure overload hypertrophy studies using genetic mouse models that prevent calcineurin-NFAT activation have confirmed its crucial role in this form of hypertrophic remodeling.^{8–11} Whether calcineurin is involved in myocyte hypertrophy post-MI, or whether inhibition of this type of hypertrophic remodeling may have therapeutic potential, remains uncertain. To address these questions, we pursued a genetic approach to evaluate the functional significance of calcineurin in post-MI cardiac remodeling, using a genetic mouse model with mild myocyte-restricted overexpression of MCIP1, which was previously shown to efficiently antagonize cardiac hypertrophy in response to an activated calcineurin transgene, after pressure overload or β -adrenergic stimulation.^{10,12} Accordingly, MCIP1-TG mice and their wild-type littermates were randomized to receive either a large transmural LV infarct or sham operation. We confirmed increased calcineurin activation and substantial hypertrophy in the LV 4 weeks after MI in WT mice, whereas TG mice were protected against both characteristics. Remarkably, MCIP1-TG mice also demonstrated preservation of LV geometry and contractility, improved survival, a diminution of β -MHC expression, and a pronounced reduction in interstitial collagen deposition in the remote myocardium. These findings suggest that calcineurin activation is crucial for maladaptive post-MI remodeling.

Materials and Methods

Animals and Surgical Procedures

All protocols were approved by institutional guidelines. All surgeries and subsequent analyses were performed in a blinded fashion for genotype. Eight-week-old mice from both sexes carrying the human MCIP1 transgene under the control of the α MHC promoter (an order of magnitude overexpression of MCIP1 at the mRNA level) in a C57BL/6 background (TG)¹⁰ and their nontransgenic littermates (WT) were anesthetized with 2.4% isoflurane and placed in a supine position on a heating pad (37°C). Animals were intubated with a 19G stump needle and ventilated with room air using a MiniVent mouse ventilator (Hugo Sachs Elektronik, Germany; stroke volume 250 μL , respiratory rate 210 breaths per minute). MI was induced by permanent ligation of the left coronary artery (LCA) with a 6-0 prolene suture. Occlusion of the LCA was confirmed under a dissecting microscope (Olympus SZ30, Japan) by discoloration of the ischemic area. Sham-operated animals underwent the same procedure without occlusion of the LCA.

Transthoracic Echocardiography

Three days and 4 weeks after MI, the mice were reanesthetized with 2.4% isoflurane and placed on a heating pad (37°C). Echocardiography was performed using a Sonos 5500 ultrasound machine (Hewlett-Packard) with a 12-MHz transducer applied parasternally to the shaved chest wall. For optimal resolution, the transducer was covered with a surgical latex finger filled with ultrasound transmission gel to obtain a standoff of 0.5 to 0.7 cm and used at a depth setting of 2 cm. The transducer was maneuvered to obtain both live

2-dimensional (2-D) images in a parasternal long-axis and short axis view and 2-D guided M-mode tracings crossing the anterior wall, LV cavity, and posterior wall. Images were stored on an optical disk and analyzed offline using EnConcert Image Diagnosis Application (Philips, Netherlands). All parameters were measured on both live 2-D images and M-mode tracings and averaged from four cardiac cycles.

Invasive Pressure Measurement

After the animals were anesthetized with urethane, a 1.4F high-fidelity micromanometer catheter (Millar Instruments) was introduced into the right carotid artery, advanced into the left ventricle, and pressure measurements were performed as described previously.¹³

Northern Blot Analysis

Northern blot analysis for MCIP1 was performed with 20 μg of total RNA in each lane and probed in Ultrahyb (Ambion) with a ³²P-labeled DNA fragment encompassing the 5' exon 4 splice variant of murine MCIP1 (Radprime, Invitrogen). Northern blot analysis for ANF, β -MHC, and GAPDH was performed as previously described.¹⁴ Signals were detected using a phosphor imaging screen (Biorad), and quantified with Quantity 1 software (Biorad).

Immunoprecipitation and Western Blot Analysis

To detect LV calcineurin A β abundance, 500 μg LV extract was immunoprecipitated with a pan-calcineurin A subunit antibody (AB1695, Chemicon), and probed with an anti-calcineurin A β antibody (sc-6124, Santa Cruz). To analyze the phosphorylation status of NFAT, 500 μg of LV protein extract was immunoprecipitated with either anti-NFATc1 (sc-13033, Santa Cruz) or anti-NFATc3 (sc-8321, Santa Cruz), and Western blotted with an anti-phosphoserine antibody (61-8100, Zymed). Immunoreactivity was detected with Chemiluminescence (ECL, Amersham Pharmacia Biotech).

Calcineurin Activity Assay

Phosphatase activity was measured using the calcineurin (PP2B) Assay kit (cat No. 20700, Calbiochem) according to the manufacturer's instructions. Calcineurin activity was measured as the dephosphorylation rate of a synthetic phosphopeptide substrate (RII peptide).

Histological Analysis

Four weeks after MI, all animals were anesthetized, and the hearts were arrested in diastole and perfused antegradely at physiological pressures with PBS containing 1 mg/mL sodium nitroprusside and 5% formalin. Heart tissue was fixed in 3.7% formaldehyde, cut either (1) longitudinally and perpendicular to the infarcted area and aortic root or (2) transversally, and embedded in paraffin. Sections (4 μm) were cut and stained with AZAN, laminin, hematoxylin and eosin (H&E), or Sirius red.¹⁵ Longitudinal AZAN-stained sections were used to determine infarct size. A computerized morphometric system (Quantimet 570, Leica) was utilized to calculate the percentage infarcted tissue of total left ventricular tissue. Laminin- and Sirius red-stained sections were used to determine myocyte hypertrophy or to visualize interstitial/perivascular collagen amount, respectively. Myocyte fiber size was assessed by digital surface measurement of approximately 300 cells per animal in four to six animals per group. Only myofibers with a centrally positioned nucleus were included. The amount of collagen was determined as the percentage of left ventricular tissue stained positive for Sirius red. Because the presence of collagen was more intense toward the apex, measurements were presented for both the whole septum and the apical half of the septal wall.

Statistical Analysis

The results are presented as mean \pm SEM. Statistical analyses were performed by using INSTAT 3.0 software (GraphPad) and ANOVA followed by Tukey's post hoc test when appropriate. Statistical significance was accepted at a value of $P < 0.05$.

Results

MCIP1 Overexpression Attenuates Postinfarction-Induced Calcineurin Activation

After MI, the heart undergoes an adaptive response that is accompanied by eccentric hypertrophy of the noninfarcted myocardium. Although calcineurin is activated and intimately involved in pressure overload-induced hypertrophy,^{8,9,12,16} it is uncertain whether calcineurin is also activated during postinfarction remodeling.

To explore this, calcineurin (PP2B) activity was first measured as the dephosphorylation rate of a synthetic phosphopeptide substrate. Calcineurin activity was elevated by 35% in the remote myocardium of WT-MI mice ($P<0.05$). In contrast, total calcineurin activity was decreased in TG-MI hearts by 26% compared with WT-MI (Figure 1A). LV Calcineurin $A\beta$ abundance has previously been shown to correlate with LV PP2B activity.¹⁷ Calcineurin $A\beta$ immunoreactivity was increased by 19% in WT-MI compared with the corresponding sham group ($P<0.05$), whereas this increase amounted to only 6% TG-MI animals (NS; Figure 1B).

Because activated calcineurin dephosphorylates members of the NFAT family, which in turn drive expression of the MCIP1 exon 4 splice isoform via an upstream cluster of NFAT binding sites,¹⁸ the phosphorylation status of two cardiac NFAT members and expression level of the MCIP1 exon 4 splice isoforms was determined. As expected, endogenous NFATc1 and NFATc3 was found to be in a hyperphosphorylated state in both sham groups, consistent with relatively low calcineurin activity. In contrast, the levels of both phospho-NFATc1 as well as NFATc3 decreased by 51% and 16% ($P<0.05$) in WT-MI mice, respectively, consistent with relatively higher endogenous PP2B activity. In contrast, the levels of phosphorylated NFATc1 and NFATc3 in TG-MI mice revealed a less pronounced decrease by 16% ($P<0.05$) and 1% (NS), respectively (Figure 1C), suggestive of calcineurin inhibition.

Expression of the MCIP1 exon 4 isoform provides an additional indication of myocardial calcineurin activity in vivo (see Discussion). The MCIP1 exon 4-specific probe was designed to the untranslated 5' region of the endogenous mouse 2.2 kb mRNA and therefore does not detect transcripts from the MCIP1 transgene. Northern blot analysis indicated an approximate 9-fold induction of the 5' exon 4 splice variant of MCIP1 expression after MI in WT hearts, whereas in TG-MI hearts, only a 2-fold induction was observed (Figure 1D and Discussion). Collectively, these results indicate that calcineurin is activated in the LV after MI, and that MCIP1 overexpression efficiently counteracts the activation characteristics of cardiac calcineurin.

MCIP1 Overexpression Attenuates LV Hypertrophy After MI

No significant difference in infarct size was observed between MCIP1-TG animals and their nontransgenic littermates (WT-MI $41\pm 3\%$ versus TG-MI $38\pm 4\%$; Figure 2A).

A more prominent increase in the HW/BW ratio was observed in the WT-MI group (48%) compared with MCIP1-TG MI mice (24%; $P<0.05$). The hypertrophic re-

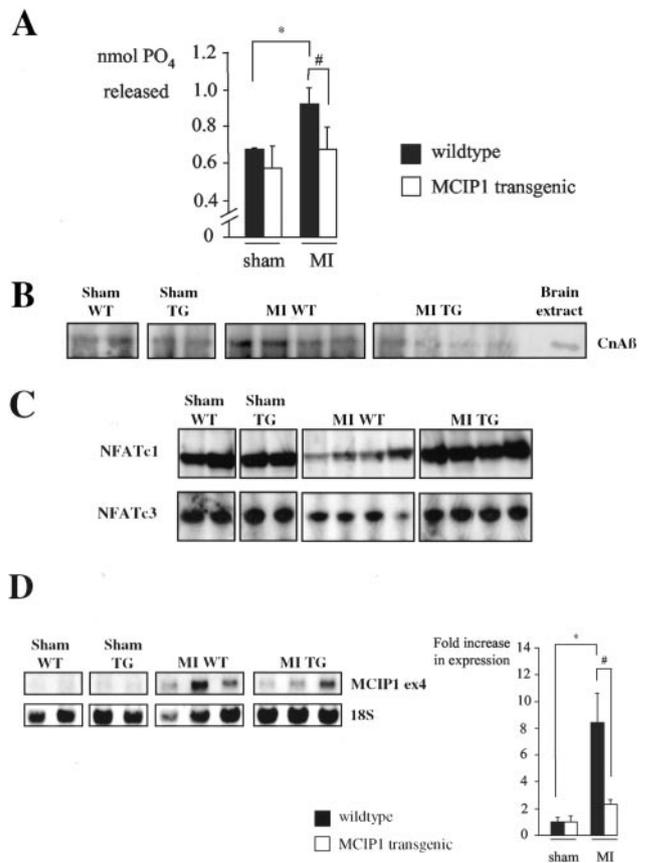


Figure 1. MCIP1 antagonizes postinfarction induced calcineurin activation. A, Cardiac extracts for the indicated groups were assayed for Cn activity, expressed as nmol released phosphate. MCIP1 transgenic mice displayed lower Cn activity both under basal conditions and after infarction. Data are expressed as mean \pm SEM from $n=3$ to 5 animals per group. * $P<0.05$ vs sham-operated group, # $P<0.05$ vs WT-MI group. B, Representative Western blot demonstrating Cn β expression for indicated groups. C, Representative Western blot for the level of hyperphosphorylated NFATc1 and NFATc3. Data demonstrate decreased phospho-NFAT levels after MI in wild-type mice, whereas MCIP1 transgenic mice have less pronounced decreases in phospho-NFAT levels compared with corresponding sham group. D, Representative Northern blot using a probe specific for the exon 4 splice variant of murine MCIP1 on LV tissue demonstrates significantly increased expression after MI in WT mice compared with sham-operated animals. As a control for loading, a probe for 18S ribosomal RNA was used. Data are expressed mean \pm SEM from $n=4$ to 7 animals per group. * $P<0.05$ vs sham operated group; # $P<0.05$ vs WT-MI group.

modeling was more pronounced in the LV and this was reflected in LVW/BW and LVW/TL ratios, which both showed a 77% and 78% increase WT-MI mice, and only a 36% and 41% increase in the MCIP1-TG groups, respectively (Table 1 and Figure 2B). Measurement of septal wall thickness in histological sections further supported these findings (WT-MI 1.1 ± 0.1 mm versus TG-MI 0.8 ± 0.2 mm; $P<0.05$). Interestingly, in both genotypes, females exhibited less pronounced hypertrophy than their male littermates (data not shown). Atrial weight (AW) and right ventricular weight (RVW) increased to a similar extent in both WT-MI and MCIP1-TG animals (Table 1).

Laminin-staining demonstrated an $81\pm 2\%$ increase of the cross-sectional area of myocytes in the spared myocardium in

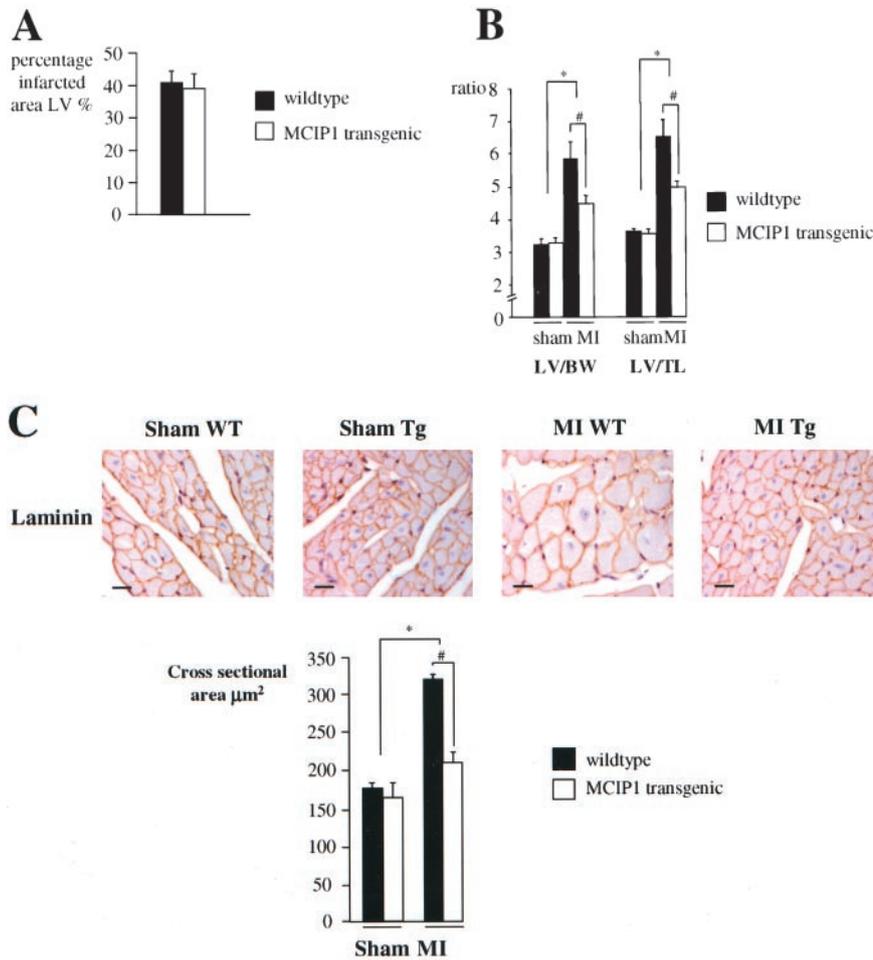


Figure 2. Attenuated hypertrophy after MI in MCIP1 transgenic mice. **A**, Morphometric analyses of AZAN sections indicate no significant difference in infarct size between the wild-type and MCIP1 transgenic mice (n=6 in both groups). **B**, After MI, an increase in LVW in the wild-type mice is evident (n=10), which is significantly blunted in the MCIP1 transgenic mice (n=12). **P*<0.05 vs sham-operated group; #*P*<0.05 vs WT-MI group. **C**, Laminin staining reveals a significant increase in cardiomyocyte size after MI compared with the sham-operated animals, which is more pronounced in wild-type animals compared with MCIP1 transgenic animals (n=4 in both sham groups, n=6 in both MI groups). Bar=0.1 mm. Quantification of cross-sectional area of myofibers from indicated groups shows significant attenuation of myocyte hypertrophy in MCIP1 transgenic mice. **P*<0.05 vs sham-operated group; #*P*<0.05 vs WT-MI group.

the WT-MI group, whereas TG-MI mice only demonstrated a $29 \pm 7\%$ increase relative to corresponding, noninfarcted, control groups (Figure 2C). Conclusively, these findings indicate that MCIP1 overexpression protects the myocardium against postinfarction hypertrophy.

Preservation of Cardiac Geometry and Function in MCIP1 TG Mice After MI

To examine the impact of MCIP1-mediated attenuation of postinfarction hypertrophy on hemodynamic behavior, all groups were subjected to 2-D and M-mode echocardiography

TABLE 1. Morphological Characteristics in Wild-Type and MCIP1 TG Mice After Sham Operation and 4 Weeks After Myocardial Infarction

	Sham		Myocardial Infarction		Percent Change TG Versus WT 4 Weeks After MI
	WT	TG	WT	TG	
n	13	12	10	12	
HW, mg	114±2	112±4	169.6±9*	143.7±5*†	-20.5
LVW, mg	77±2	76±5	137±10*	106±6*†	-38.5
RVW, mg	19.5±2	21.8±3	28.1±2*	27.3±3	-18.8
Lung weight, mg	150±4	153±3	162±5	152±4	NS
Liver weight, mg	909±33	880±34	1042±50	981.5±49	NS
Atrial weight, mg	10.9±0.8	10.5±0.5	15.7±2.2	15.9±1.4	NS
TL, mm	21.5±0.1	21.9±0.2	21.5±0.2	21.7±0.1	NS
BW, g	23.8±0.7	22.9±0.4	23.9±1.0	23.5±0.6	NS
HW/BW, mg/g	4.8±0.1	4.9±0.1	7.1±0.4*	6.1±0.3*†	-23.4
HW/TL, mg/mm	5.3±0.1	5.1±0.1	7.9±0.5*	6.6±0.3*†	-19.7

Data are expressed as mean±SEM. TG indicates transgenic; HW, heart weight; LVW, left ventricular weight; RVW, right ventricular weight; TL, tibial length; and BW, body weight.

**P*<0.05 vs corresponding sham group; †*P*<0.05 vs WT postinfarction group.

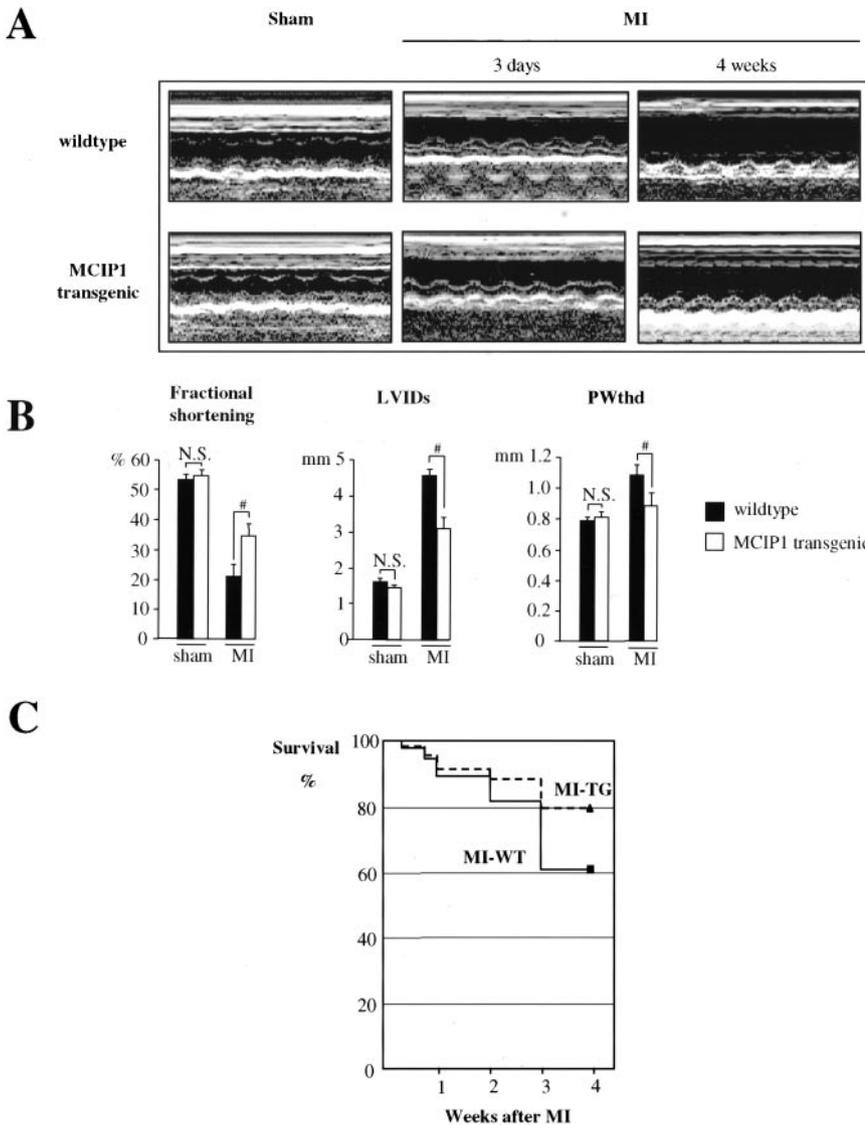


Figure 3. Preservation of cardiac function in MCIP1 transgenic mice after MI. A, Representative M-mode images of sham or infarcted wild-type and MCIP1 transgenic mice 3 days and 4 weeks after MI. Data demonstrate comparable initial LV dilation in both groups, with progressive dilation and loss of contractile behavior in wild-type mice, which was substantially attenuated in MCIP1 transgenic mice. B, Bar graph representations of FS, LVIDs, and PWthd indicate that the presence of the MCIP1 transgene attenuates functional and morphological deterioration after MI (n=5 in both sham groups; n=7 to 8 in MI groups). #P<0.05 vs WT-MI group. C, Early post-MI mortality was comparable between wild-type and transgenic mice subjected to MI, but became significantly higher for wild-type mice in the last 3 weeks before euthanasia (WT-MI, n=51; TG-MI, n=43).

3 days and 4 weeks after MI, because hypertrophic remodeling in mice starts 3 days after the ligation of the coronary artery.¹⁹ Representative images of M-mode recordings are shown in Figure 3A. Already 3 days after infarct, an increase in left ventricular internal diameter (LVID) and decrease in contractility was evident in both MI groups compared with sham-operated animals, with no functional or geometric differences between WT-MI and TG-MI (Table 2). In contrast, 4 weeks after MI, a thickening of the posterior wall in diastole (PWthd) and a further increase in LVID was visible in WT-MI mice, suggestive of progressive LV dilation (Table 2). These geometric changes were accompanied by a further functional deterioration in WT-MI mice as indicated by progressive decreases in fractional shortening (FS) and ejection fraction (EF) compared with the corresponding sham group (Figure 3B). The presence of the MCIP1 transgene resulted in a significant reduction of PWthd and LVID in response to MI, and a preservation of FS (Table 2 and Figure 3B) and EF (Table 2).

To further evaluate the hemodynamic profile of all experimental groups, invasive pressure measurements were per-

formed. A relatively lower systolic baseline function was observed in sham-TG compared with sham-WT mice (Table 3). At present, we have no explanations for this difference. More importantly, the response of both genotypes to MI was clearly different. In the WT-MI group, a dramatic decrease in LV dp/dt_{max} , LV dp/dt_{min} , LVESP, and LVDP was observed ($P<0.05$). In contrast, TG-MI mice showed a marked preservation of these parameters, and this level of preservation correlates well with the echocardiographic observations (Table 3 and Figure 3).

Taken together, these results indicate that MCIP1 overexpression specifically alters the late phase of post-MI remodeling rather than the acute response to the infarct itself and efficiently counteracts the geometric and functional deterioration after MI.

Overexpression of MCIP1 Improves Survival After Myocardial Infarction

The survival rates of WT and MCIP1-TG mice were evaluated up to 4 weeks after MI. The survival of WT-MI mice was 89% and 61% at 1 and 4 weeks after surgery, whereas

TABLE 2. Echocardiographic Characteristics in Wild-Type and MCIP1 TG Mice After Sham Operation and After Myocardial Infarction

	Sham		3 Days After MI		4 Weeks After MI		Percent Change TG Versus WT 3 Days After MI	Percent Change TG Versus WT 4 Weeks After MI
	WT	TG	WT	TG	WT	TG		
n	7	8	7	6	12	11		
HR	400±18	402±17	388±12	390±15	406±11	401±12	NS	NS
PWthd, mm	0.68±0.05	0.67±0.06	0.73±0.05	0.73±0.02	1.09±0.08*	0.82±0.04†	NS	-38
PWths, mm	1.39±0.07	1.29±0.06	1.31±0.03	1.28±0.07	1.50±0.18	1.30±0.07	NS	NS
LVIDd, mm	3.52±0.12	3.21±0.07	4.44±0.13*	4.34±0.13*	5.56±0.14*	4.69±0.24*†	NS	-12
LVIDs, mm	1.68±0.08	1.52±0.04	3.11±0.11*	3.10±0.10*	4.36±0.12*	2.98±0.12*†	NS	-64
% EF	62.75±2.3	58.5±2.8	35.5±3.4*	32.5±1.5*	26.0±2.5*	36.3±4.8*†	NS	21
% FS	52.2±2.0	52.6±1.4	29.3±1.4*	29.7±1.3*	21.6±2.3*	36.9±1.8*†	NS	29

Data are expressed as mean±SEM. TG indicates transgenic; PWthd, posterior wall thickness in diastole; PWths, posterior wall thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; FS, left ventricular fractional shortening calculated as (LVIDd-LVIDs)/LVIDd; and EF, left ventricular ejection fraction.

* $P<0.05$ vs corresponding sham group; † $P<0.05$ vs WT 4-week postinfarction group.

MCIP1-TG MI mice displayed a survival of 90% and 79%, respectively (Figure 3C). Differences in survival became evident in the last 3 weeks before euthanasia (Figure 3C) and was more pronounced in male littermates (data not shown). These observations correlate well with the fact that infarct sizes were equal in both groups, and that infarct healing is completed within 7 days after MI in mice.¹⁵ The combined observations suggest that MCIP1 overexpression has no influence on the early process of infarct healing, but attenuates hypertrophic LV remodeling that starts ≈3 to 7 days after MI in mice and improves postinfarction survival.

Attenuated Interstitial Collagen Deposition After Calcineurin Inhibition in MI

H&E staining confirmed the presence of extensive myofiber hypertrophy and disarray in WT-MI mice (Figure 4A), accompanied by a patchy presence of cellular infiltrates in the interstitial compartment (indicated by arrowheads). These histological alterations in response to infarction were nearly absent in TG-MI hearts similar to both sham-operated groups (Figure 4A). In the noninfarcted area, reactive fibrosis, such as perivascular and interstitial fibrosis, was observed in a gradient with more pronounced presence of collagen deposition toward the infarct. WT-MI mice exhibited the most severe fibrotic alterations, both expressed as percentage of the complete septum (10%) as well as the lower apical half of the septal wall (16%), and this was substantially attenuated in

MCIP1-TG MI mice (Figure 4B). These results indicate that the presence of the MCIP1 transgene led to a significant reduction of the major structural alterations of the remote myocardium in the postinfarcted heart.

Cardiac Gene Expression in Infarcted Hearts With Calcineurin Inhibition

We examined the gene expression profile of genes activated during postinfarction hypertrophy. At 4 weeks after MI, an induction of both ANF and β -MHC was observed in WT-MI hearts relative to corresponding sham-operated mice, indicating the activation of a fetal cardiac gene program. ANF expression was similar in both TG-MI and WT-MI hearts. In contrast, the induction of β -MHC expression was significantly blunted in TG-MI hearts compared with WT-MI hearts (Figure 5). These results indicate that the transcriptional response of the MCIP1 transgenic animals is different than that of wild-type animals during MI-induced LV remodeling.

Discussion

Suppression of Postinfarction Hypertrophic Remodeling by MCIP1

The involvement of calcineurin as a transducer of prohypertrophic signals in the heart has gradually gained more acceptance. The use of genetically modified mice with reduced or enhanced calcineurin activity in the heart has

TABLE 3. Hemodynamic Characteristics in Wild-Type and MCIP1 TG Mice After Sham Operation or Myocardial Infarction

	Wild-Type		Percent Change Versus Corresponding Sham Group	MCIP1 Transgenic		Percent Change Versus Corresponding Sham Group
	Sham	MI		Sham	MI	
n	4	5		5	6	
LV dP/dt _{max} , mm Hg · s ⁻¹	9338±481	6202±903	-34	5524±886	5402±830	-2
LV dP/dt _{min} , mm Hg · s ⁻¹	-6645±672	-4681±517	-30	-5128±651	-4832±630	-6
LVDp, mm Hg	93.5±4.9	72.9±4.7	-22	66.4±6.2	66.2±5.8	0
LVESP	94.6±5.3	76.9±4.3	-19	67.1±6.1	67.3±6.0	0
LVEDP	1.1±1.0	4.1±1.5	372	0.8±0.3	3.1±0.6	388

Data are expressed as mean±SEM. LV dP/dt_{max} indicates first maximal derivative of left ventricular pressure; LV dP/dt_{min}, first minimal derivative of left ventricular pressure; LVDp, left ventricular developed pressure; LVESP, left ventricular end-systolic pressure; and LVEDP, left ventricular end-diastolic pressure.

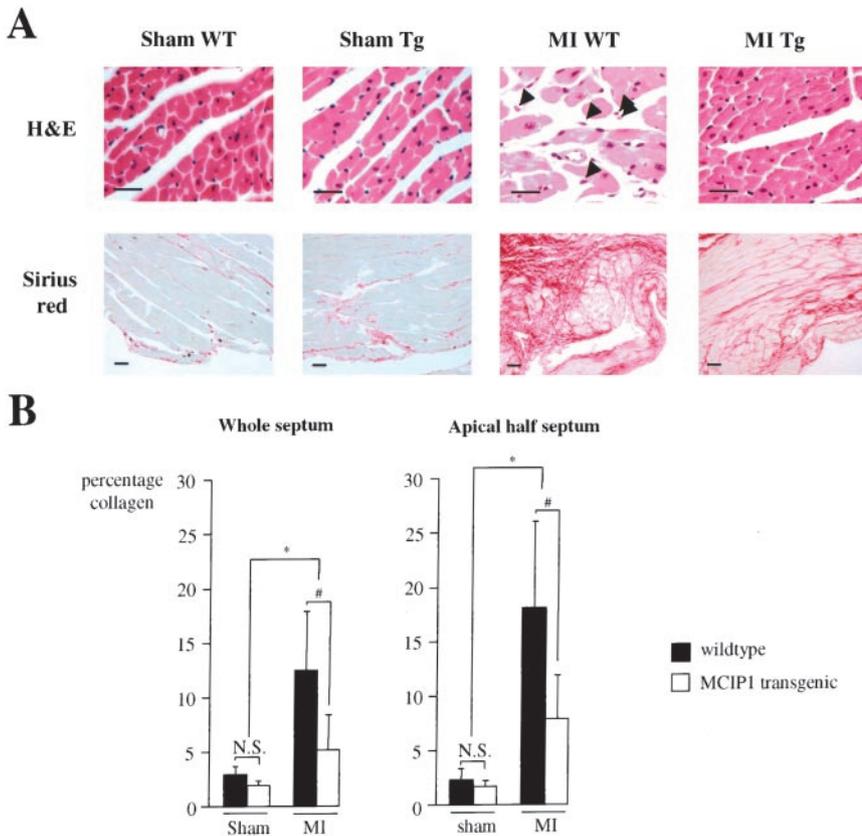


Figure 4. MCIP1 transgenic animals are protected against MI-induced structural remodeling. **A**, Representative images of histological staining for the indicated groups. Top, H&E-stained images; Bottom, Sirius red-stained images (bar=0.1 mm). H&E-stained images reveal remarkable myocyte hypertrophy, myofiber disarray, and cellular infiltrates (arrowheads) in a section from a representative postinfarcted wild-type mouse. In contrast, MCIP1 transgenic mice are largely protected against these structural alterations. Sirius red staining indicates low levels of collagen deposition in the sham-operated mouse hearts from both phenotypes, a massive accumulation of fibrillar collagen (interstitial and perivascular fibrosis) after infarction in wild-type mice, which is substantially attenuated in MCIP1 transgenic mice. **B**, Tissue content of fibrillar collagen in septum (remote myocardium) is significantly more upregulated in wild-type animals than in MCIP1 transgenic mice, a difference that is even more pronounced in the lower half of the septal wall ($n=2$ in both sham groups; $n=6$ in both MI groups). * $P<0.05$ vs sham-operated group; # $P<0.05$ vs WT-MI group.

confirmed the pathological role of this enzyme in cardiac muscle cell biology.^{8–11,16,20} Most studies, however, have focused on the function of calcineurin in hypertrophy development after acute increases in loading conditions (eg, aortic banding procedures) or agonist infusion protocols. The potential role of calcineurin in LV remodeling after ischemic loss of functional myocardium has remained ill defined. In view of the increasing proportion of heart failure patients with coronary artery disease as a primary etiology,¹ elucidation of the regulatory pathways controlling postinfarction remodeling will have both fundamental and therapeutic avenues.

Preceding the present study, three groups have used rat models of myocardial infarction (MI) in combination with systemic delivery of the calcineurin inhibitor cyclosporin A (CsA) to address the role of calcineurin in post-MI remodeling. All three groups found a correlation between CsA administration and reduced postinfarct hypertrophy.^{21–23} CsA toxicity and potential calcineurin-independent effects of CsA can confound interpretation of these studies.²⁰ Our findings from the present study using MCIP1 transgenic mice are in agreement with the rat studies insofar as the attenuation of hypertrophy is concerned. However, in contrast to some of the CsA studies, long-term prognosis of the MCIP1 transgenics is improved compared with wild-type rather than in the CsA-treated rats, which showed a more rapid progression toward heart failure. The MCIP1-TG mice allowed us to achieve cardiac-specific inhibition of calcineurin without the concern of secondary drug effects or systemic toxicity.²⁰

Quantification of calcineurin phosphatase activity from tissue lysates using biochemical assays is problematic, due to the dynamic, calcium/calmodulin-dependent regulation of activity and the sensitivity of calcineurin to oxidation of the Fe-Zn active center, and does not reflect the proportion of the enzymatic calcineurin pool that is actually in an active state. We could detect both increased phosphatase activity and more abundant presence of the calcineurin A β isoform after MI in wild-type mice. We also used the phosphorylation status of endogenous NFAT and expression levels of the NFAT-responsive exon 4 splice variant of MCIP1 as criteria to monitor calcineurin activity.¹⁰ These assays were in line with increased calcineurin activation in the postinfarcted heart. The present study confirms the earlier documented calcineurin activation profile in the postinfarcted LV, but our interpretation of its value in LV remodeling differs in several respects from other studies.

Postinfarction Hypertrophy Is Maladaptive in Nature

Myocardial hypertrophy is traditionally viewed as a long-term adaptive response of the cardiac muscle to either altered mechanical loading conditions (eg, resulting from valvular disease or hypertension) or decreased performance due to loss of contractile units (eg, after MI).⁴ After this interpretation, increased wall thickness serves as the means to restore wall stress in line with the law of Laplace.⁴ Recent insights into the particulars of the hypertrophic phenotype, however, have demanded a more nuanced interpretation of this phenomenon of “compensatory hypertrophy.” First, ventricular hypertrophy is demonstrably a risk factor for cardiovascular mortality

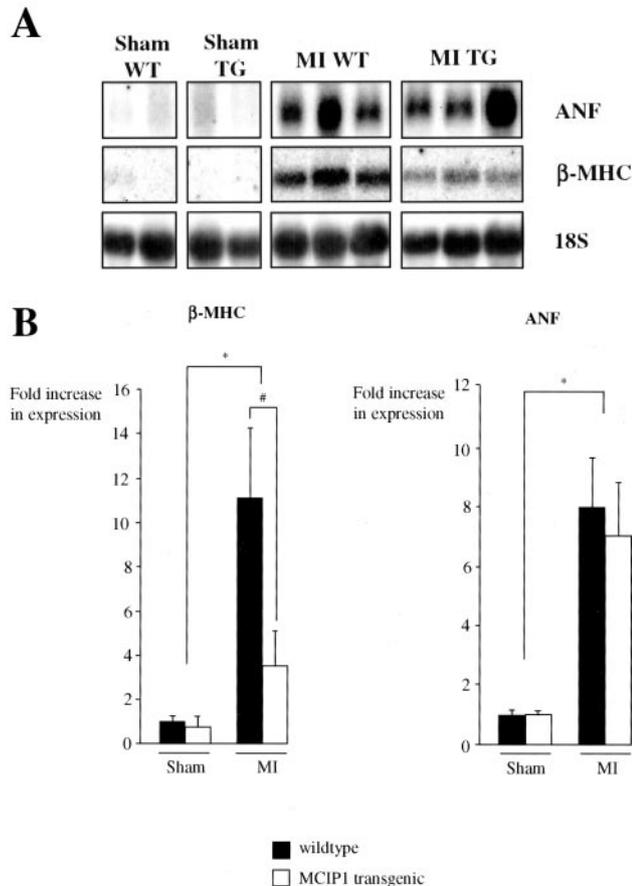


Figure 5. MI-induced fetal gene expression is differentially affected by MCIP1 overexpression. A, Representative Northern blot for ANF (top panels), β -MHC (middle panel), and 18S ribosomal RNA from indicated experimental groups. Data indicate strong upregulation of ANF and β -MHC after infarction in wild-type mice and lower β -MHC expression in postinfarcted transgenic animals. B, Quantification of Northern blot analyses indicates significant upregulation of ANF and β -MHC gene expression after MI in wild-type mice. Only MI-evoked elevation of β -MHC is significantly blunted in the MCIP1 transgenic mice. * $P < 0.05$ vs sham-operated group; # $P < 0.05$ vs WT-MI group.

in humans.⁵ Second, beyond just increased mass, the specific long-term transcriptional responses to increased load entail a myriad of quantitative and qualitative changes in cardiac gene expression that are reminiscent of fetal cardiac myocytes. In patients with cardiac failure, functional improvement related to treatment with β -blockers is correlated with beneficial changes in myocardial gene expression, most prominently exemplified by a correction in the mRNA expression level of the β -MHC gene.²⁴ In the present study, we also noted a pronounced decrease in β -MHC gene expression and no relative change in ANF expression in infarcted transgenic mice compared with their wild-type counterparts (Figure 5). Of note, in the original description of MCIP1 overexpressing mice,¹⁰ this divergence between ANF versus β -MHC fetal gene expression was also evident. ANF and its receptor have been reported to possess potent antihypertrophic activity,^{25,26} whereas recombinant human BNP has been shown to improve decompensated congestive heart failure in patients.²⁷ It will become of interest to investigate the specific contribution

of natriuretic peptides in the calcineurin-NFAT axis. Conclusively, Laplace's law, although conceptually sound, does not take into account any qualitative alterations of the wall, and only incompletely explains the phenotypic particulars of heart enlargement.

Third, several studies using genetically engineered mice with markedly blunted growth responses to pressure overload appear to be protected from adverse effects of stress signaling and heart failure progression.^{12,28} In fact, Esposito et al²⁸ subjected normal and hypertrophy-resistant mice to aortic constriction and observed that hemodynamic function and overall cardiac geometrics were better preserved in hypertrophy-resistant mice than in wild-type mice, despite the inability of the transgenic mice to correct wall stress. Hence, the value of normalizing wall stress through hypertrophic myocardial growth is dispensable for the preservation of cardiac function in the face of a long-term hemodynamic burden.²⁹ The findings in the present study fortify the conclusions that postinfarction-, and load-induced reactive signaling and hypertrophic remodeling share fundamental similarities and may be equally deleterious in nature.²⁹ One outspoken observation in postinfarcted MCIP1 overexpressing mice was their marked inhibition of interstitial collagen deposition. It is important to note, that the MCIP1 transgene is targeted exclusively to the cardiac muscle cell population, which suggests a crucial role and hierarchy for the cardiomyocyte population in governing cardiac fibroblast proliferation and collagen production through paracrine regulation of latter cell type.

Dual Roles of MCIP1 in Calcineurin Signaling

Although we interpret the protective effects of MCIP1 on post-MI cardiac pathology to reflect the reduction in calcineurin activity in the hearts of MCIP1-TG mice, it should also be pointed out that our recent studies have revealed positive and negative effects of MCIP1 on calcineurin activity. It is clear that high levels of MCIP1 expression can inhibit calcineurin activity through direct association with the catalytic subunit of the enzyme. Paradoxically, however, MCIP1 knockout mice, which are viable, show a reduction in cardiac calcineurin activity and a diminished hypertrophic response to pressure overload and chronic adrenergic stimulation.³⁰ We have attributed the latter findings to a permissive role of MCIP1 in activation of calcineurin, possibly via a chaperone or protein folding function of MCIP1. It is also formally possible that MCIP1 evokes activities through cellular effectors in addition to calcineurin. Further confirmation of the precise roles of MCIP1 and calcineurin in post-MI hypertrophy and remodeling will be provided by studies of these processes in calcineurin-TG mice and in knockout mice lacking calcineurin and MCIP1.

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