Selective Vascular Endothelial Protection Reduces Cardiac Dysfunction in Chronic Heart Failure

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Background**—**Chronic heart failure (CHF) induces endothelial dysfunction in part because of decreased nitric oxide ($NO^·$) production, but the direct link between endothelial dysfunction and aggravation of CHF is not directly established. We previously reported that increased NO production via inhibition of protein tyrosine phosphatase 1B (PTP1B) is associated with reduced cardiac dysfunction in CHF. Investigation of the role of endothelial PTP1B in these effects may provide direct evidence of the link between endothelial dysfunction and CHF.

Methods and Results**—**Endothelial deletion of PTP1B was obtained by crossing LoxP-PTP1B with Tie2-Cre mice. CHF was assessed 4 months after myocardial infarction. In some experiments, to exclude gene extinction in hematopoietic cells, Tie2-Cre/LoxP-PTP1B mice were lethally irradiated and reconstituted with bone marrow from wild-type mice, to obtain mouse with endothelial-specific deletion of PTP1B. Vascular function evaluated ex vivo in mesenteric arteries showed that in wild-type mice, CHF markedly impaired NO-dependent flow-mediated dilatation. CHF-induced endothelial dysfunction was less marked in endoPTP1B$^{-/-}$ mice, suggesting restored NO production. Echocardiographic, hemodynamic, and histological evaluations demonstrated that the selectively improved endothelial function was associated with reduced left ventricular dysfunction and remodeling, as well as increased survival, in the absence of signs of stimulated angiogenesis or increased cardiac perfusion.

Conclusions**—**Prevention of endothelial dysfunction, by endothelial PTP1B deficiency, is sufficient to reduce cardiac dysfunction post myocardial infarction. Our results provide for the first time a direct demonstration that endothelial protection per se reduces CHF and further suggest a causal role for endothelial dysfunction in CHF development. **(*Circ Heart Fail*. 2016;9:e002895. DOI: 10.1161/CIRCHEARTFAILURE.115.002895.)**

Key Words: bone marrow ■ endothelium ■ heart failure ■ myocardial infarction ■ nitric oxide

A large body of experimental and clinical evidence has accumulated to demonstrate that chronic heart failure (CHF) is associated with profound endothelial dysfunction, including impaired endothelial nitric oxide (NO) production.[1–7] It is also generally assumed that endothelial dysfunction per se contributes to aggravate CHF, as supported by observations that such dysfunction is associated with increased morbidity/mortality in patients with CHF.[8,9] Indeed, reduced coronary $NO^·$ production most likely impairs cardiac perfusion, favors inflammation, and affects cardiac contractility and excitation-contraction coupling.[10] In parallel, reduced $NO^·$ production/bioavailability in peripheral resistance arteries favors vasoconstriction thus contributing to the increased peripheral resistance known to aggravate CHF via increased cardiac afterload.[4] Thus, prevention of endothelial dysfunction is considered an important goal of current CHF treatments.

See Clinical Perspective

Paradoxically, although the aggravating effects of endothelial dysfunction in CHF have been largely suggested, this has never been directly demonstrated. Conversely, there is no clear evidence that prevention of endothelial dysfunction per se may in turn result in reduced CHF. Indeed, although many pharmacological treatments of CHF prevent endothelial dysfunction,[2,11,12] the contribution of the endothelium to their beneficial effects in CHF is unclear. In fact, reduction of CHF may per se secondarily reduce endothelial dysfunction, as demonstrated for example with heart rate–reducing agents[13] that are devoid of direct endothelial effects but lead to endothelial protection in CHF.[14] Thus, a direct demonstration of these links requires the development of approaches with "selective" endothelial targeting in CHF.

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From the Inserm (Institut National de la Santé et de la Recherche Médicale) U1096, Department of Pharmacology, Rouen, France (J.M., M.B., E.G., N.B., J.-P.H., O.B., L.N., P.M., J.M., V.R.); Normandy University, Institute for Research and Innovation in Biomedicine, Rouen, France (J.M., M.B., E.G., N.B., J.-P.H., L.N., P.M., V.R.); and Inserm (Institut National de la Santé et de la Recherche Médicale) U905, Department of Immunology, Rouen, France (O.B., J.M.).

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Correspondence to Vincent Richard, PhD, Inserm U1096, Rouen University Medical School, 22 Blvd Gambetta, 76183 Rouen, France. E-mail vincent.richard@univ-rouen.fr

Our group discovered a new approach for endothelial protection in CHF, based on inhibition of the protein tyrosine phosphatase 1B (PTP1B).[3] PTP1B is a ubiquitously distributed protein that dephosphorylates various tyrosine kinase receptors, notably insulin, leptin, and vascular endothelial growth factor receptors, modulates immune signaling, and also acts either as tumor suppressor or tumor promoter depending on the cellular context.[15] Thus, although no results have emerged to date from clinical trials, PTP1B inhibitors are currently tested in patients with obesity or type 2 diabetes mellitus and in patients with breast cancer.

Interestingly, we demonstrated that chronic pharmacological inhibition or whole body gene deletion of PTP1B in CHF not only prevented endothelial dysfunction but also improved left ventricular (LV) function and decreased adverse LV remodeling.[16] However, because PTP1B is expressed in many cells other than the endothelium, the exact role of PTP1B-mediated endothelial protection in these beneficial cardiac effects remains unknown. We thus developed a mouse model of selective endothelial PTP1B deficiency to directly assess the role of endothelial protection in CHF.

Methods

Animals and Surgery

All animal experiments were ethically approved by a certified review board according to French and EU legislation (authorization number 01307.01).

Homozygous loxP PTP1B (later referred to as PTP1B$^{f/f}$, C57BL/6J strain, CD45.2)[17] were obtained from Dr Neel (University of Toronto, Canada), and were crossed with transgenic mice expressing Cre recombinase under the control of the Tie2 promoter (The Jackson Laboratory, Bar Harbor, ME). These Tie2-Cre(+)PTP1B$^{f/f}$ mice, later named Tie2PTP1B$^{-/-}$, were compared with Tie2-Cre(−) PTP1B$^{f/f}$ littermates, used as wild-type (WT) controls lacking PTP1B deletion.

The Tie2-Cre approach induces gene deletion not only in endothelial but also in hematopoietic cells.[18,19] Thus, to assess the respective roles of PTP1B deletion in the endothelial versus hematopoietic cells and especially restrict the ablation of PTP1B to the endothelium, Tie2PTP1B$^{-/-}$ mice were lethally irradiated (10 Gy, Faxitron) 2 months before induction of myocardial infarction (MI) and grafted with 20 million bone marrow (BM) cells from CD45.1 C57/B6 mice (Jackson Laboratory; for chimerism monitoring, see below), to obtain mice with selective endothelial PTP1B deficiency (endoPTP1B$^{-/-}$). They were compared with 3 groups: (1) mice with selective deletion of PTP1B in the hematopoietic compartment (CD45.1 C57/B6 mice irradiated and grafted with BM from Tie2PTP1B$^{-/-}$ mice, referred to as Tie2-BM-PTP1B$^{-/-}$), (2) mice without PTP1B deletion (CD45.2 irradiated and grafted with BM from CD45.1 mice referred to as BM-WT, and (3) Tie2PTP1B$^{-/-}$ irradiated and grafted with BM from Tie2PTP1B$^{-/-}$ mice.

At the end of the experiment, BM cell suspensions from transplanted mice were stained with anti-CD45.1 and anti-CD45.2 (APC-Cy5.5; Becton Dickinson, Franklin Lakes, NJ). The percentage of BM chimerism was evaluated by flow cytometry (FACS Canto II; Becton Dickinson).

MI was induced by left coronary artery ligation in male mice (body weight, 22–25 g)[3,16] anesthetized with 3.6 mg/kg xylazine IP followed by continuous isoflurane 2% inhalation (1.5 mL/min; Baxter) during artificial ventilation, and sedated with buprenorphine (0.5 mg/kg). Anesthesia and sedation were controlled by monitoring heart rate, and by performing paw pinch reflex and corneal reflex tests.

Procedures for evaluation of cardiac function, remodeling and perfusion, as well as endothelial function, western blotting, polymerase chain reaction (PCR), and immunohistochemistry are described in the Data Supplement.

Statistical Analysis

Data are presented as mean±SEM. For vascular functional studies, comparisons were performed using 2-factor repeated measurement ANOVA. Survival was analyzed by Mantel Cox test. All other comparisons were performed by nonparametric Kruskal–Wallis analysis followed by Dunn post hoc test. $P<0.05$ was considered significant.

Results

Endothelial PTP1B Deletion

In mesenteric arteries, Tie2PTP1B$^{-/-}$ mice displayed significant mRNA expression of the Cre-truncated form of PTP1B, whereas the corresponding reverse transcriptase-PCR signal was low in WT mice. Tie2PTP1B$^{-/-}$ mice also displayed a markedly decreased expression of the WT form of PTP1B (Table 1). Immunohistochemistry showed that PTP1B was present in the mesenteric artery endothelium of WT but not in the Tie2PTP1B$^{-/-}$ mice (Figure 1A).

Vascular Function

In WT sham mice, the stepwise increase in intraluminal flow induced a progressive increase in mesenteric artery diameter (ie,

Table 1. Mesenteric Artery Expression of Various Genes Assessed by Reverse Transcriptase-Polymerase Chain Reaction

	WT Sham (n=9)	Tie2PTP1B$^{-/-}$ Sham (n=10)	WT CHF (n=10)	TiePTP1B$^{-/-}$ CHF (n=11)
WT PTP1B	0.77±0.02	0.26±0.05*	0.80±0.05	0.26±0.05*
PTP1B Cre-truncated	0.30±0.03	0.78±0.03*	0.22±0.02	0.78±0.10*
eNOS	0.49±0.04	0.60±0.04	0.50±0.06	0.44±0.05
iNOS	0.48±0.14	0.47±0.10	0.92±0.23	0.99±0.37
nNOS	0.48±0.06	0.48±0.05	0.59±0.04	0.47±0.05
CD45	0.52±0.14	0.62±0.08	0.43±0.07	0.56±0.13
ICAM-1	0.46±0.07	0.53±0.06	0.52±0.08	0.52±0.09
VCAM-1	0.49±0.09	0.56±0.07	0.56±0.09	0.46±0.09

No differences were detected between sham and CHF for any of the parameters. CHF indicates chronic heart failure; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; ICAM, intercellular adhesion molecule; nNOS, neuronal nitric oxide synthase; PTP1B, protein tyrosine phosphatase 1B; VCAM, vascular cell adhesion molecule; and WT, wild-type.

*$P<0.001$ vs corresponding WT.

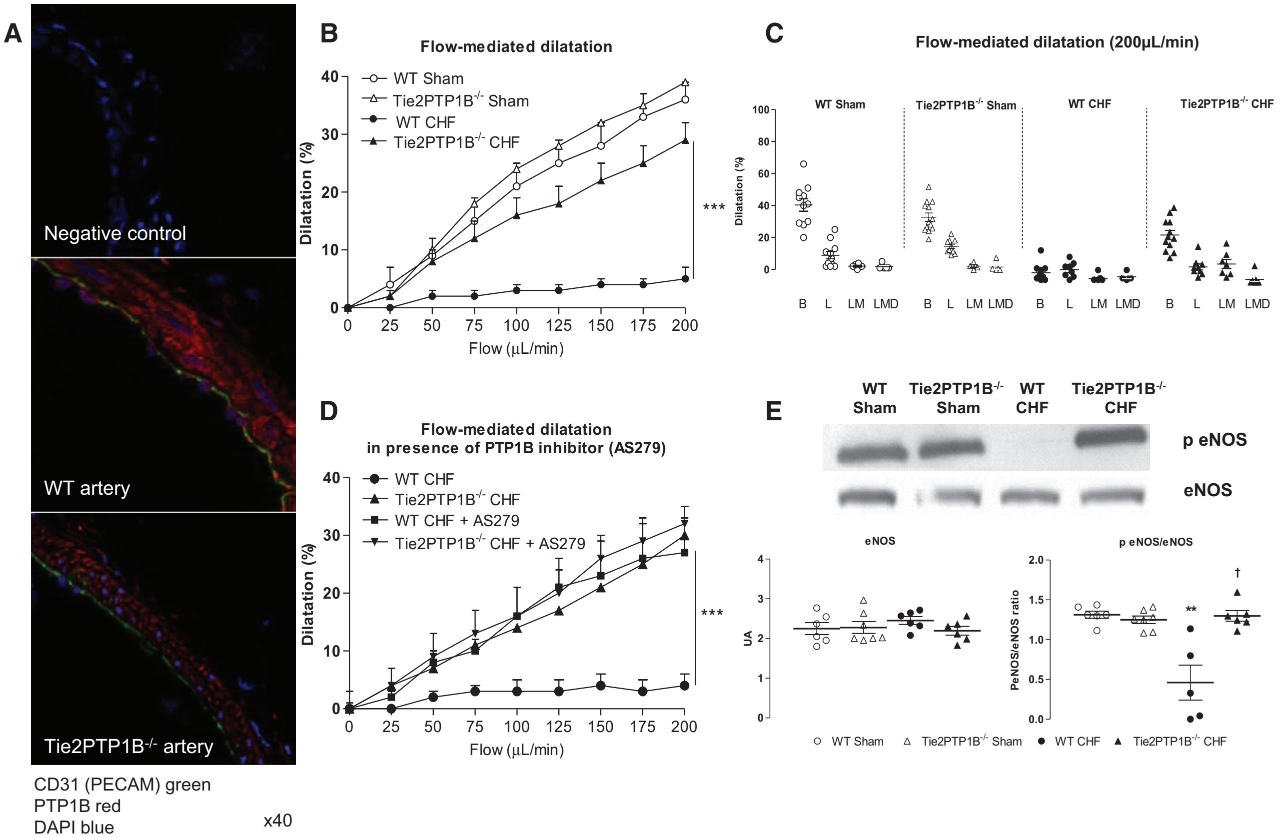

Figure 1. A, Immunohistological evidence of the presence of protein tyrosine phosphatase 1B (PTP1B; red) in the endothelium of wild-type (WT) but not Tie2PTP1B$^{-/-}$ mice. Negative control (**top**) corresponds to incubation without the secondary antibody. **B**, Effect of chronic heart failure (CHF) and PTP1B deletion on mesenteric artery flow-mediated dilatation (FMD; n=9–12 animals per group). ***P<0.001. **C**, Effect of in vitro incubation with the nitric oxide synthase inhibitor L-NNA alone (n=8–11 animals per group) or in association with the cytochrome P450 inhibitor MSPPOH (n=5–7) and the cyclooxygenase inhibitor diclofenac (Diclo; n=5–7). **D**, Effect of acute in vitro incubation with the PTP1B inhibitor (PTP1Bi) AS279 ($10^{-5\ \mathrm{mol/L}}$; n=5–6). ***P<0.001. Values for **B**–**D** are mean± SEM. **E**, Representative Western blot data of total endothelial nitric oxide synthase (eNOS) or P Ser1177-eNOS, and mean±SEM eNOS and P Ser1177-eNOS/eNOS ratio (n=5–7) in mesenteric arteries exposed to flow at 200 µL/min for 2 min. **P<0.01 vs WT sham; †P<0.05 vs WT CHF. B indicates base; D, Diclo; L, L-NNA; and M, MSPPOH.

flow-mediated dilatation [FMD]; Figure 1B) that was markedly reduced by NG-nitro-L-arginine (L-NNA) and virtually abolished by L-NNA+N-methylsulfonyl-6-(2-propargyloxyphenyl)-hexanamide (MSPPOH; Figure 1C), suggesting that FMD was mostly mediated by NO· with a moderate part caused by epoxyeicosatrienoic acids. In WT, CHF mice presented a near complete abolition of FMD, without alteration of the vascular dilatory responses to acetylcholine (Figure 2A) or the NO· donor SNP (Figure 2B). In these WT CHF mice, L-NNA did not significantly inhibit the remaining response, suggesting that the impaired FMD was entirely because of reduced NO· production/bioavailability (Figure 1C). Furthermore, arteries from WT CHF displayed an almost complete abolition of eNOS Ser^{1177} phosphorylation, in the absence of changes of total eNOS (Figure 1E for western blot, Table 1 for PCR).

In sham mice, FMD was similar in WT and Tie2PTP1B$^{-/-}$; however, the inhibitory effect of L-NNA appeared slightly less marked in Tie2PTP1B$^{-/-}$ than in WT, whereas in both the cases, the L-NNA-resistant response was abolished by MS-PPOH, suggesting that endothelial PTP1B deficiency tended to increase the epoxyeicosatrienoic acids–mediated component of relaxation. In Tie2PTP1B$^{-/-}$ mice with CHF, FMD was markedly increased compared with WT CHF, and was comparable to that observed in sham (Figure 1B). This was accompanied by a restored inhibitory effect of L-NNA (Figure 1C) and of phosphorylated eNOS (Figure 1E), suggesting that it was associated with a restoration of the impaired NO production.

Furthermore, although acute in vitro PTP1B inhibition markedly increased FMD in WT CHF mice, this effect was absent in Tie2PTP1B$^{-/-}$ mice with CHF (Figure 1D), suggesting that the beneficial arterial effect of this inhibition is indeed because of blockade of the endothelial PTP1B. Compared with WT, Tie2PTP1B$^{-/-}$ CHF mice did not show any changes in the dilatory response to acetylcholine (Figure 2A) or to the NO· donor SNP (Figure 2B). Neither CHF nor PTP1B deletion affected the arterial expression of inducible nitric oxide synthase, neuronal nitric oxide synthase, CD45, ICAM-1, and VCAM-1 (Table 1).

Echocardiography

In WT mice, CHF was associated with a marked and progressive LV dilatation (Figure 3A–3C), and a 70% decrease in LV fractional shortening (FS, Figure 3D), accompanied by an inverted *E*/*A* ratio (<1) suggestive of impaired LV diastolic function (Figure 3E).

PTP1B deletion did not affect LV diameters, FS, or *E*/*A* ratio in sham mice. In contrast, compared with sham CHF

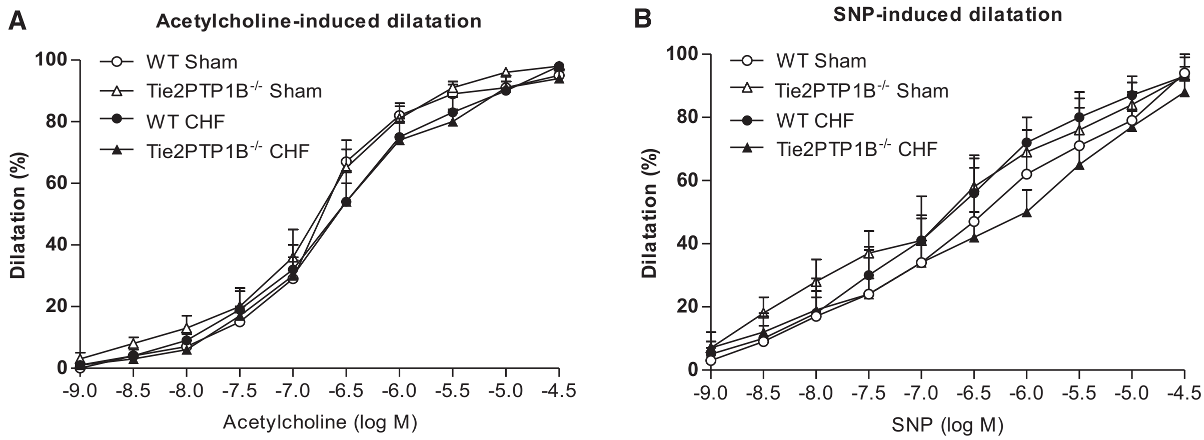

Figure 2. Effect of chronic heart failure (CHF) and protein tyrosine phosphatase 1B (PTP1B) deletion on (**A**) acetylcholine-mediated and (**B**) sodium nitroprusside–mediated vasodilatation of mesenteric arteries (n=9–12). Values are mean± SEM.

mice, Tie2PTP1B$^{-/-}$ CHF displayed a marked reduction in LV dilatation (Figure 3A–3C), a 75% increase in FS (Figure 3D), and a normalization of *E*/*A* ratio (Figure 3E) showing a significant improvement of both LV systolic and diastolic functions.

LV Hemodynamics

In WT, CHF decreased diastolic and systolic arterial pressures (Figure 4A and 4B), as well as LV end-systolic pressure and end-systolic pressure/volume relationship (Figure 4D–4F), demonstrating impaired LV systolic function. In parallel, these CHF WT displayed a significant increase in LV end-diastolic pressure and a nonsignificant increase in end-diastolic pressure/volume relationship (Figure 4C–4E), demonstrating diastolic dysfunction.

In sham mice, Tie2PTP1B$^{-/-}$ did not show any differences with WT in arterial or LV pressures. In contrast, compared with WT CHF, Tie2PTP1B$^{-/-}$ CHF displayed an increase in arterial pressures (significant only for systolic pressure, Figure 4A and 4B), a nonsignificant increase in LV end-systolic pressure

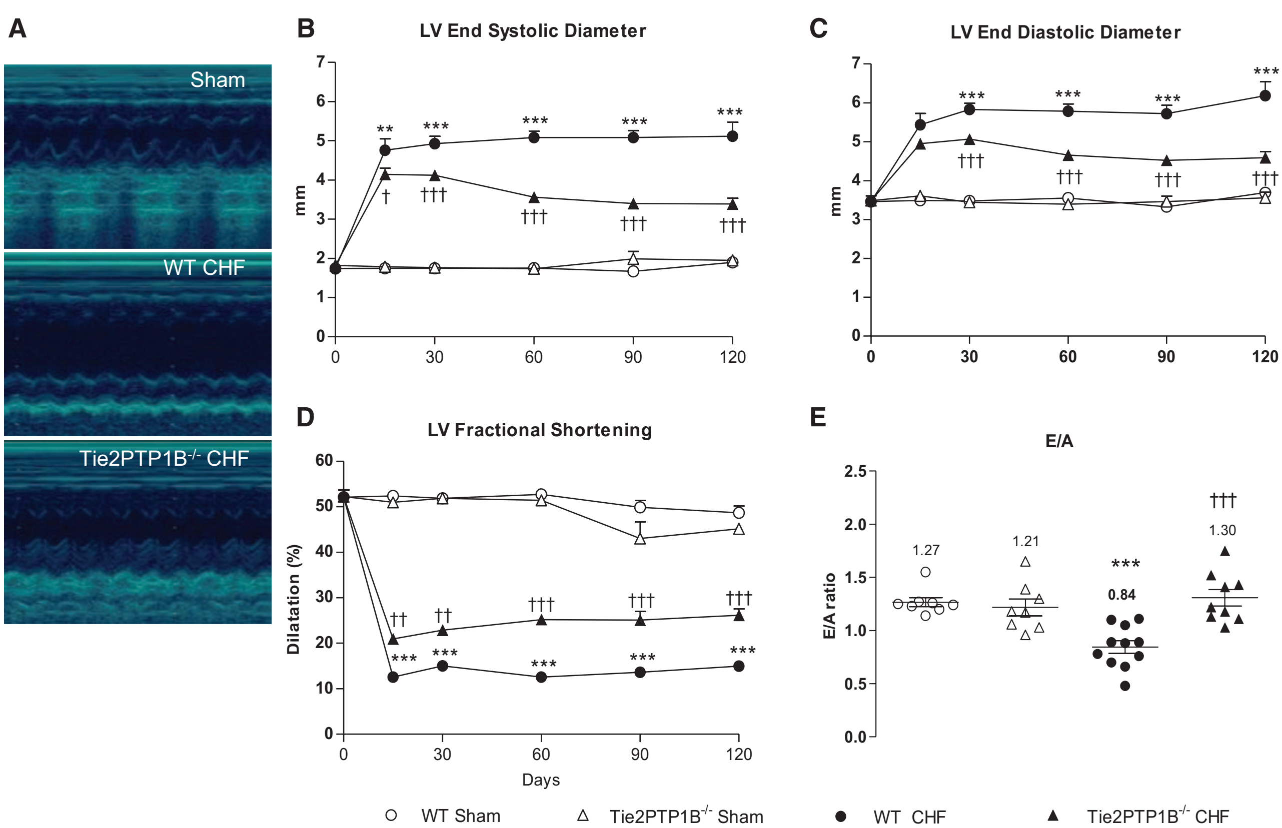

Figure 3. A, Representative echocardiographic tracings obtained in wild-type (WT) sham, WT chronic heart failure (CHF), and Tie2PTP1B$^{-/-}$ mice. **B**–**D**, Evolution with time of left ventricular (LV) systolic diameter (**B**), LV diastolic diameter (**C**), and LV fractional shortening (**D**), evaluated by echocardiography. **E**, *E*/*A* ratio evaluated by transmitral pulsed Doppler at 4 months post-myocardial infarction (MI). Values are mean±SEM (n=8–14 per group). ***P*<0.01 and ****P*<0.001 vs WT sham; †*P*<0.05, ††*P*<0.01, and †††*P*<0.001 vs WT CHF.

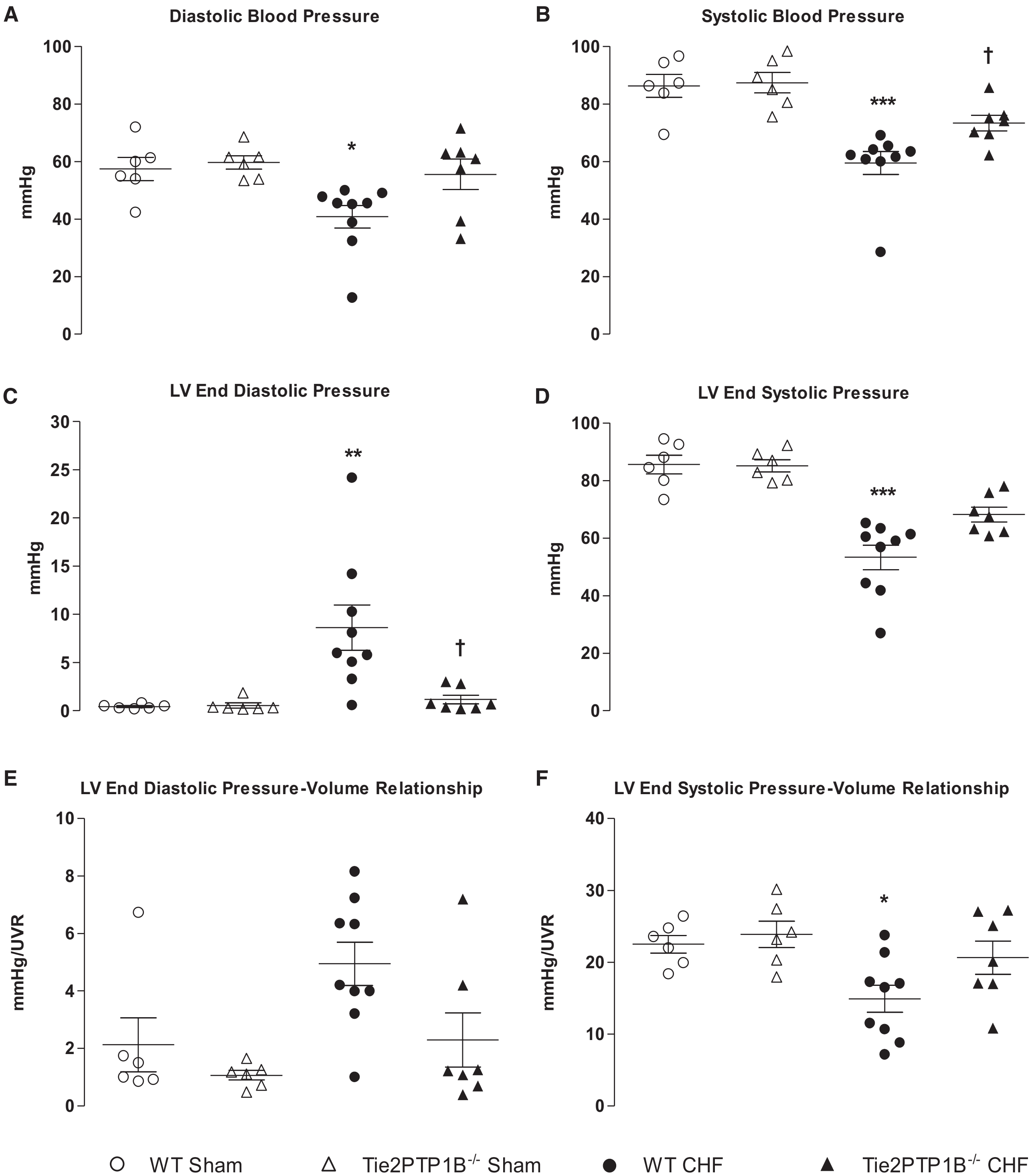

Figure 4. Mean±SEM diastolic (**A**) and systolic (**B**) carotid blood pressure, left ventricular (LV) end-diastolic (**C**) and end-systolic (**D**) pressures, and LV end-diastolic (**E**) and end-systolic (**F**) pressure volume relationship (n=6–9 animals per group). *P<0.05, **P<0.01, and ***P<0.001 vs wild-type (WT) sham; †P<0.05 vs WT chronic heart failure (CHF).

(Figure 4D), and a significant decrease in LV end-diastolic pressure (Figure 4C). Furthermore, Tie2PTP1B$^{-/-}$ mice were partly prevented against the CHF-induced decrease in LV end-systolic pressure/volume relationship (Figure 4F) and the increase in LV end-diastolic pressure/volume relationship (Figure 4E); however, these effects did not reach statistical significance.

Cardiac Remodeling, Perfusion, and Gene Expression

Infarct size was not significantly different between WT (17.9±0.1% of LV, n=8) and Tie2PTP1B$^{-/-}$ mice (20.7±0.1% of LV, n=8). Compared with sham, CHF WT mice displayed significant increases in both LV and RV weights (Figure 5A

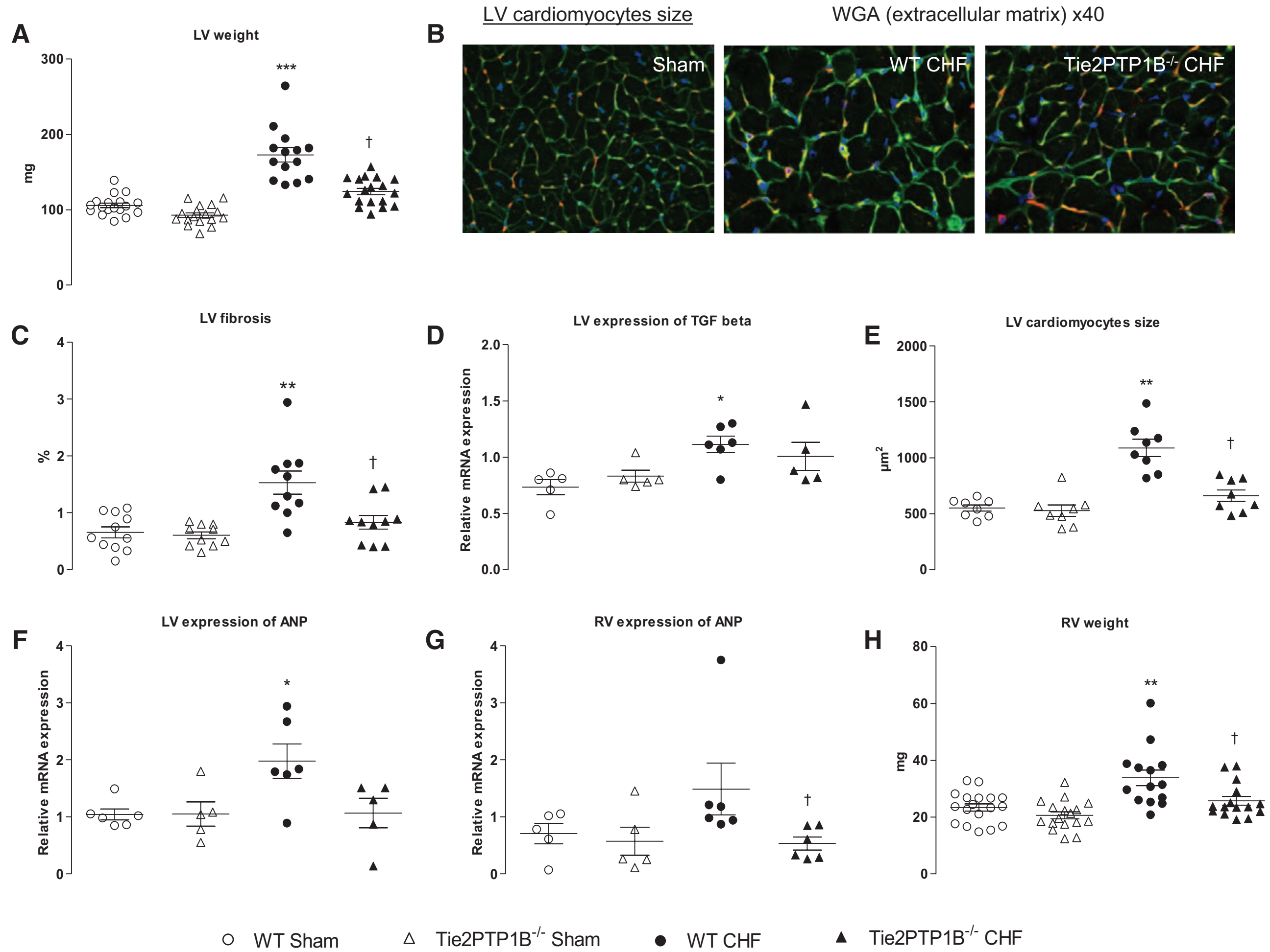

Figure 5. A, Mean±SEM left ventricular (LV) weight. **B**, Representative images of cardiac sections stained with wheat germ agglutinin (WGA) in wild-type (WT) sham, WT chronic heart failure (CHF), and Tie2PTP1B$^{-/-}$ CHF mice. (**C–H**), LV collagen density (**C**), mRNA expression of tumor growth factor β (TGFβ) (**D**), LV cardiomyocyte size (**E**), LV expression of atrial natriuretic peptide (ANP) (**F**) and brain natriuretic peptide (**G**), and right ventricular (RV) weight (**H**). Values are mean±SEM from 8 to 11 animals per group for LV weight, fibrosis and cardiomyocytes size and RV weight and 5 to 6 animals per group for LV expression of TGFβ, ANP, and RV expression of ANP. **P*<0.05, ***P*<0.01, and ****P*<0.001 vs WT sham; †*P*<0.05 vs WT CHF.

and 5H), LV cardiomyocyte sizes (Figure 5B and 5E), and collagen density (Figure 5C). CHF also increased LV mRNA expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP; Figure 5F and 5G; Table 2) as well as matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), and neuronal nitric oxide synthase (nNOS) in the absence of detectable changes in tumor growth factor β (TGFβ; Figure 5D; Table 2), eNOS, inducible nitric oxide synthase, CD45, F4/80, αmyosin heavy chain, and βmyosin heavy chain (Table 2).

Compared with WT, Tie2PTP1B$^{-/-}$ CHF mice showed significantly smaller increases in these parameters of LV and RV hypertrophy and fibrosis, demonstrating reduced adverse cardiac remodeling, associated with reduced LV mRNA expression of ANP, BNP, MMP2, and MMP9 (although not significant for MMP2), in the absence of changes in the expression of the other genes tested (Table 2).

Neither CHF nor PTP1B deletion affected LV capillary density (capillary/myocyte ratio: WT sham 1.09±0.08, n=11; Tie2PTP1B$^{-/-}$ sham: 1.12±0.05, n=11; WT CHF 1.29±0.06, n=10; Tie2PTP1B$^{-/-}$ CHF 1.31±0.06, n=11) or LV perfusion (mL·min^{-1}·g^{-1}: WT sham 11.0±0.7, n=8; Tie2PTP1B$^{-/-}$ sham: 11.2±0.6, n=9; WT CHF 9.7±0.5, n=11; Tie2PTP1B$^{-/-}$ CHF 9.7±0.5, n=16).

Bone Marrow Transplantation

In Tie2PTP1B$^{-/-}$ CD45.2 mice irradiated and grafted with CD45.1 (WT) BM, 95±3% of hematopoietic cells were CD45.1 positive, whereas in CD45.1 mice irradiated and grafted with Tie2PTP1B$^{-/-}$ CD45.2 BM, 96±2% of hematopoietic cells were CD45.2 positive demonstrating the excellent efficacy of the transplantation (Figure 6A).

WT (CD45.2) mice reconstituted with CD45.1 BM (WT-BM) displayed similar vascular and cardiac responses to CHF than nonirradiated WT CHF mice, including a near complete abolition of FMD (Figure 6B), similar LV hypertrophy (Figure 6C) and fibrosis (Figure 6E), and similar decrease in LV FS (Figure 6D). Importantly, WT mice reconstituted with BM from Tie2PTP1B$^{-/-}$ mice also did not differ from WT CHF mice in terms of these endothelial and cardiac responses.

Table 2. Left Ventricular Expression of Various Genes Assessed by Reverse Transcriptase-Polymerase Chain Reaction

	WT Sham (n=6)	Tie2PTP1B$^{-/-}$ Sham (n=5)	WT CHF (n=8)	Tie2PTP1B$^{-/-}$ CHF (n=9)
eNOS	1.21±0.04	1.07±0.09	0.90±0.07	1.12±0.03
iNOS	1.05±0.09	1.09±0.10	0.76±0.09	1.06±0.06
nNOS	1.13±0.09	1.21±0.13	0.78±0.06*	0.93±0.13
CD45	0.83±0.07	1.30±0.21	1.36±0.33	1.60±0.19
F4/80	0.90±0.05	0.93±0.07	1.24±0.17	1.54±0.14
αMHC	1.36±0.09	1.53±0.07	1.04±0.17	1.08±0.04
βMHC	1.11±0.05	1.22±0.12	0.99±0.11	1.07±0.03
ANP	1.04±0.10	1.05±0.21	1.98±0.30†	1.07±0.26§
BNP	0.73±0.29	1.04±0.19	3.15±0.42†	1.63±0.54§
MMP2	0.66±0.05	0.73±0.06	1.64±0.19‡	1.17±0.09
MMP9	0.45±0.14	0.96±0.13	1.31±0.14†	0.71±0.11§
TGFβ	0.81±0.10	0.83±0.05	1.03±0.08	1.01±0.12

αMHC indicates αmyosin heavy chain;βMHC, βmyosin heavy chain; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHF, chronic heart failure; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; LV, left ventricular; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; nNOS, neuronal nitric oxide synthase; PTP1B, protein tyrosine phosphatase 1B; TGF, transforming growth factor β; and WT, wild-type.

*$P<0.05$, †$P<0.01$, ‡$P<0.001$ vs WT sham; §$P<0.05$ vs WT CHF.

In contrast, Tie2PTP1B$^{-/-}$ mice grafted with BM from WT mice (ie, selective endothelial deficiency) displayed signs of cardiovascular protection similar to those of nonirradiated Tie2PTP1B$^{-/-}$ mice described above, including fully restored mesenteric FMD (Figure 6B), markedly and significantly increased LV FS (Figure 6D) as well as decreased LV hypertrophy (Figure 6C) and fibrosis (Figure 6E). These effects were virtually identical to those observed in Tie2PTP1B$^{-/-}$ grafted with BM from Tie2PTP1B$^{-/-}$ mice. Together, these results demonstrate that the protective endothelial and cardiac effects observed in Tie2PTP1B$^{-/-}$ mice were entirely because of deletion of PTP1B in vascular endothelial cells and not in hematopoietic cells.

Survival

In WT, CHF markedly decreased survival (46% at 4 months). Mortality occurred essentially between 1 week and 2 months post-MI. Compared with WT, Tie2PTP1B$^{-/-}$ had markedly reduced mortality (86%, Figure 7A). A similar effect on survival was observed in endoPTP1B$^{-/-}$ mice (Figure 7B).

Discussion

The present study, performed in mouse model of MI-induced CHF, shows that selective deletion of PTP1B in the endothelium is not only associated with markedly reduced endothelial dysfunction (ie, restored flow-dependent, NO·-mediated dilatation, and eNOS phosphorylation in mesenteric arteries) but is also accompanied by reduced CHF as shown by the improved LV function, hemodynamics, and remodeling, as well as a markedly increased survival. To the best of our knowledge, this is the first direct demonstration that prevention of endothelial dysfunction per se leads to a reduction of CHF.

We[3,16] and others[20–22] previously revealed that PTP1B is a target for the prevention of endothelial dysfunction, in the context of diabetes mellitus, obesity, and CHF. Many mechanisms may indirectly contribute to this protective effect, especially in the context of chronic in vivo inhibition or gene deletion. However, the fact that in CHF, endothelial function (FMD) and eNOS phosphorylation may be improved by acute in vitro incubation of isolated arteries with a PTP1B inhibitor[3] strongly suggested that a large part of the protective effects directly involve the endothelium (eg, restoration of phosphorylation pathways of eNOS activation) and is not the indirect consequence on the endothelium of improved CHF. In this context, the fact that in the present study, selective endothelial PTP1B deficiency restored endothelial function and flow-induced eNOS phosphorylation to the same extent as that observed after long-term pharmacological inhibition or global gene deletion[16] reinforces the view of the crucial role of endothelial PTP1B in the aggravation of endothelial dysfunction. It must be noted that under some conditions, reduced endothelial dysfunction may also be indirectly caused by nonendothelial PTP1B deletion, as shown for example in obese mice with hepatic PTP1B deficiency, in which the endothelial protective effects are most likely secondary to the simultaneously improved glucose and lipid homeostasis and increased insulin sensitivity.[22] In any case, the markedly reduced endothelial dysfunction observed here in endoPTP1B$^{-/-}$ mice provides a unique situation to assess the cardiac consequences of selective endothelial protection in the context of CHF.

Endothelial protection observed in mice with endothelial PTP1B deficiency was associated with a potent reduction in the severity of CHF. This was observed in terms of echography (increased LV FS and normalized *E*/*A* ratio) and invasive LV hemodynamics. In parallel, endothelial protection also triggered profound beneficial effects on LV remodeling, demonstrated by the decreased LV dilatation assessed by

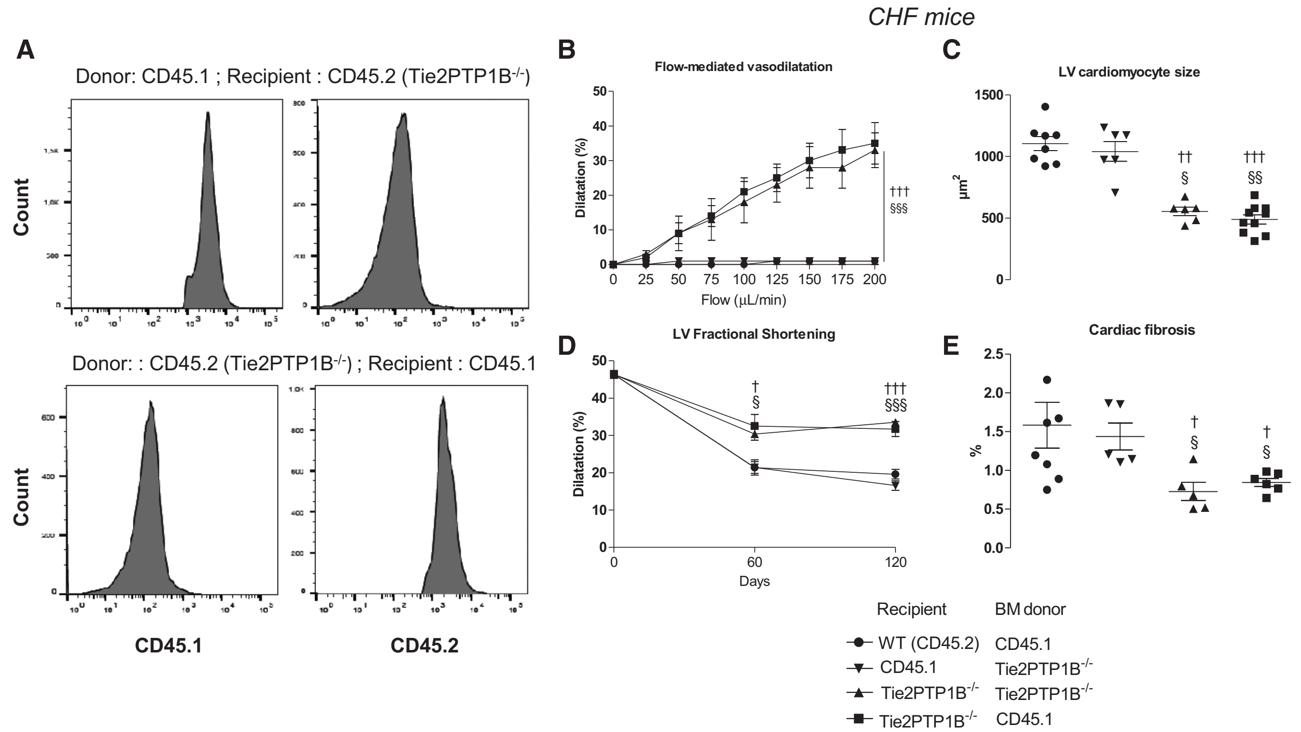

Figure 6. **A**, Flow cytometry analysis of cells expressing CD45.1 (**left**) and CD45.2 (**right**) in CD45.2 mice transplanted with CD45.1 bone marrow (BM) (**top**) or CD45.1 mice transplanted with CD45.2 BM (**bottom**). **B–E**, Mesenteric artery flow-mediated dilatation (FMD; **B**), left ventricular (LV) cardiomyocyte size (**C**), LV fractional shortening (FS; **D**), and LV fibrosis (**E**) in irradiated and transplanted chronic heart failure (CHF) mice. Values are mean±SEM from 5 to 8 animals per group. †*P*<0.05, ††*P*<0.01, and †††*P*<0.001 vs CD45.2 transplanted with CD45.1 BM; §*P*<0.05, §§*P*<0.01, and §§§*P*<0.001 vs CD45.1 transplanted with Tie2PTP1B$^{-/-}$ BM.

echography, together with decreased cardiac hypertrophy and fibrosis, and reduced cardiac ANP and BNP.

We used the Tie2-Cre approach as it is known to be effective and potent for targeted gene deletion in the endothelium. Indeed, it was associated with a profound reduction in the mRNA expression of native (WT; nontruncated) PTP1B in mesenteric arteries. Its expression was not abolished, however, most likely because of the maintained expression of PTP1B in nonendothelial vascular cells, for example, smooth muscle cells. We, however, confirmed by immunohistochemistry using an antibody directed toward the deleted (catalytic) part of the protein that the full-length PTP1B was absent from endothelial cells. This was accompanied by a strong expression of the Cre-truncated form of PTP1B assessed by reverse transcription-PCR. These results show that the Tie2-Cre approach indeed resulted in a profound PTP1B deletion in the

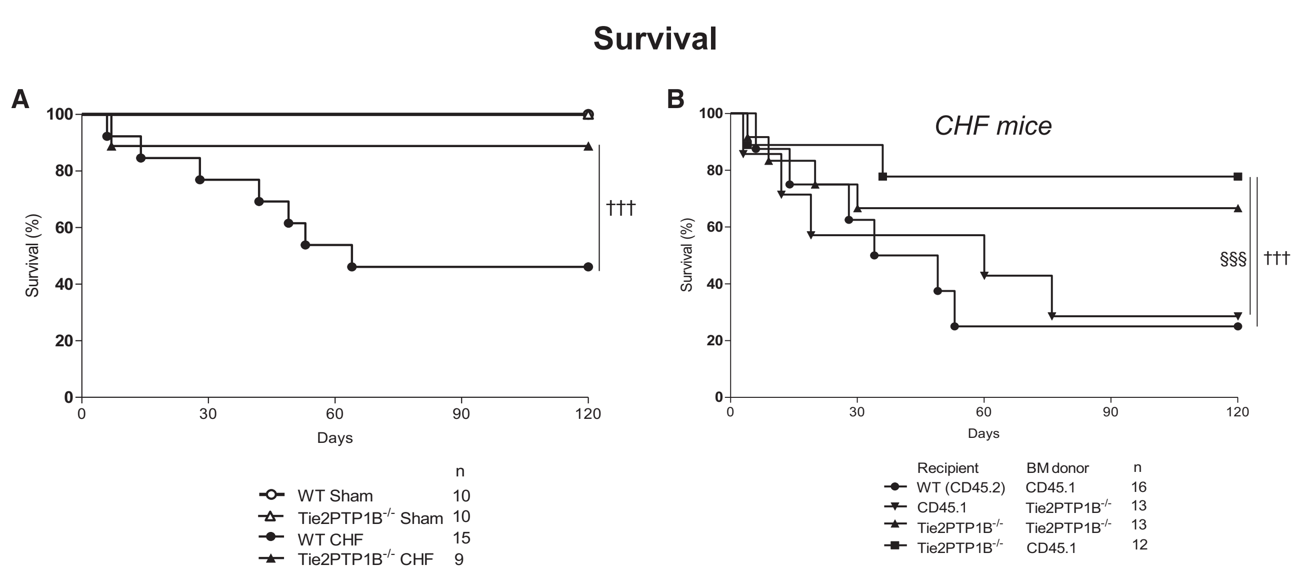

Figure 7. Evolution of survival for 4 months after myocardial infarction (MI), (**A**) in sham and chronic heart failure (CHF) wild-type (WT) or Tie2PTP1B$^{-/-}$ mice, and (**B**) in CHF mice subjected to irradiation and bone marrow (BM) transplantation. †††*P*<0.001 vs CD45.2 transplanted with CD45.1 BM; §§§*P*<0.001 vs CD45.1 transplanted with Tie2PTP1B$^{-/-}$ BM.

endothelial cells. However, although commonly used because of its potency, the Tie2 approach has the limitation that it is also associated with gene extinction in hematopoietic Tie2-expressing cells.[18,19] This was verified in our study (PTP1B mRNA expression in BM cells: WT 1.01±0.05; Tie2PTP1B$^{-/-}$ mice 0.05±0.05). Thus, to separate the effects between endothelial versus hematopoietic PTP1B deletion, and to obtain a model with selective endothelial PTP1B deficiency, we performed additional irradiation/BM transplant experiments. With this technique, we reached a high efficacy of the irradiation/BM transplantation with >95% chimerism. Importantly, we verified that this protocol did not affect endothelial or cardiac function, or the effects of CHF on these parameters. Indeed, WT mice irradiated and transplanted with WT BM had values similar to nonirradiated mice in terms of FMD, LV FS, hypertrophy, and fibrosis, both in sham and CHF mice.

Next, we demonstrated that mice with PTP1B deletion restricted to BM cells displayed no reduction in endothelial dysfunction and no improvement in cardiac function and remodeling. In contrast, mice with PTP1B deletion restricted to the endothelium (endoPTP1B$^{-/-}$) showed potent endothelial protection and reduction of cardiac dysfunction and remodeling, and these effects were similar to those observed in mice with deletion both in the endothelium and hematopoietic compartments. Thus, importantly, this demonstrates that the observed reduction of CHF is indeed entirely the consequence of PTP1B deletion in the vascular endothelium.

Immunohistochemical and PCR data obtained in arteries with endothelial PTP1B deficiency suggested that PTP1B is also present in arterial smooth muscle cells, although with a lesser expression than that of endothelial cells, as demonstrated by the two third decrease in arterial gene expression in Tie2PTP1B$^{-/-}$ mice. The exact roles of this smooth muscle form of PTP1B are unclear; however, it is unlikely to modulate arterial relaxation as suggested by the absence of changes in the responses to sodium nitroprusside in Tie2PTP1B$^{-/-}$ mice (this study) as well as in mice with global PTP1B deficiency or after pharmacological PTP1B inhibition.[16]

Increased myogenic tone of resistances arteries (possibly secondary to endothelial dysfunction) is known to contribute to increased peripheral resistance in CHF[23,24] and thus probably is an aggravating factor this disease. Thus, although we have not addressed this question, it is possible that endothelial PTP1B deletion positively modulates myogenic tone and that this contributes to the overall reduction of CHF. This hypothesis deserves to be tested in subsequent experiments.

The endothelial and cardiac effects of endothelial PTP1B deletion were associated with a significant increase in 4-month survival. This beneficial effect was observed a clinically relevant setting, because in contrast with many mouse studies in which marked mortality occurs within the first week and is low thereafter, virtually all mortality occurred in the present study after the first week post-MI. We think that this low immediate mortality is because of improved postoperative care of the animals.

We hypothesized that the reduction of CHF observed in Tie2PTP1B$^{-/-}$ mice was the consequence of the reduced endothelial dysfunction. One alternative hypothesis would be that it may be partly the consequence of a proangiogenic effect of the deletion. Indeed, we showed previously that global (whole body) PTP1B deletion in mice with MI increased cardiac vascular endothelial growth factor signaling, angiogenesis, and perfusion at 8 days post-MI.[25] Similarly, mice with endothelial PTP1B deficiency also showed increased vascular endothelial growth factor signaling, together with increased angiogenesis and arteriogenesis in a model of hindlimb ischemia, although cardiac ischemia was not studied in this study.[26] In contrast, in this study, we found no change in cardiac capillary density or MRI-based LV perfusion at 4 months post-MI. Thus, although we cannot exclude that early changes in angiogenesis or perfusion may have occurred in our study, this would suggest that the reduced CHF severity that we observed is to a large extent independent of changes in cardiac angiogenic responses.

To the best of our knowledge, our study is the first to directly address the consequences of selective reduction of endothelial dysfunction on CHF. Several studies reported the beneficial effects of eNOS overexpression in CHF;[27] however, this does not fully reproduce reduction of endothelial dysfunction, and in fact cardiac-specific eNOS overexpression is also beneficial in this setting.[28] A recent study reported reduced cardiac fibrosis and CHF in mice with endothelial P53 deficiency[29]; however, this study did not evaluate endothelial function and further was performed in a model of transverse aortic constriction that does not recapitulate all the characteristics of changes in endothelial function that can affect CHF (especially changes in cardiac afterload).

In conclusion, using mice with selective endothelial PTP1B deficiency, we demonstrated a direct link between endothelial dysfunction and aggravation of CHF. This not only further supports the concept that PTP1B inhibition is a promising target of this disease but also clearly reinforces the importance of targeting the endothelium in the treatment of CHF.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Although it is usually accepted that endothelial dysfunction contributes to symptoms and progression of chronic heart failure (CHF), the direct deleterious effect of endothelial dysfunction in this setting has not been established. Using a novel approach of selective endothelial protection (endothelial specific deletion of protein tyrosine phosphatase 1B) in a mouse model of CHF postmyocardial infarction, we demonstrated that such selective endothelial protection was sufficient to improve left ventricular function and reduce adverse remodeling. Such a direct demonstration that endothelial protection per se reduces the propensity to develop CHF suggests a causal role for endothelial dysfunction in CHF development. Thus, the endothelium should be considered as a potential target in the treatment to prevent CHF post infarction.