Pressure Overload Induces Cardiac Dysfunction and Dilation in Signal Transducer and Activator of Transcription 6–Deficient Mice

Shungo Hikoso, MD; Osamu Yamaguchi, MD, PhD; Yoshiharu Higuchi, MD, PhD; Shinichi Hirotani, MD, PhD; Toshihiro Takeda, MD; Kazunori Kashiwase, MD; Tetsuya Watanabe, MD; Masayuki Taniike, MD; Ikuko Tsujimoto, DMD; Michio Asahi, MD, PhD; Yasushi Matsumura, MD, PhD; Kazuhiro Nishida, MD, PhD; Hiroshi Nakajima, MD, PhD; Shizuo Akira, MD, PhD; Masatsugu Hori, MD, PhD; Kinya Otsu, MD, PhD

Background—Signal transducer and activator of transcription (STAT) proteins constitute a family of transcription factors that mediate many cytokine-induced responses. STAT6 is activated by angiotensin II and in rat hypertrophied hearts and in human hearts with dilated cardiomyopathy. This suggests that STAT6 may be involved in the pathogenesis of cardiac hypertrophy and heart failure. For this study we used STAT6-deficient (STAT6−/−) mice to examine the in vivo role of STAT6.

Methods and Results—STAT6−/− hearts showed no morphological, histological, or functional defects. We examined left ventricular structural and functional remodeling 1 week after thoracic transverse aortic constriction (TAC). Western blot and immunohistochemical analyses showed increased STAT6 activity after TAC in the heart of wild-type mice. STAT6−/− mice showed a significant increase in end-diastolic left ventricular internal dimension accompanied by impaired contractility compared with wild-type mice but no differences in hypertrophic parameters. The number of terminal deoxynucleotidyl transferase–mediated biotin dUTP nick-end labeling–positive myocytes after TAC had increased in STAT6−/− compared with wild-type mice. Prolonged induction of tumor necrosis factor-α (TNF-α) mRNA was observed in STAT6−/− hearts, whereas TNF-α mRNA was only transiently induced in wild-type mice. Tristetraprolin was induced after TAC in wild-type mice but not in STAT6−/− mice. Tristetraprolin reporter assay with the use of isolated neonatal cardiomyocyte indicated that the promoter was significantly activated by endothelin-1 in wild-type but not in STAT6−/− cardiomyocytes. The lack of promoter activation by endothelin-1 in STAT6−/− cardiomyocytes was rescued by forced expression of STAT6.

Conclusions—STAT6 plays a protective role against hemodynamic stress in hearts. (Circulation. 2004;110:2631-2637.)

Key Words: heart failure ■ signal transduction ■ immune system

The ability of the myocardium to adapt against hemodynamic overload or myocardial ischemia ultimately determines whether the heart decompensates and fails or instead maintains preserved function. The signal transduction pathways mediating the progression to heart failure have been studied in a variety of in vitro and in vivo animal models. These pathways include specific G-protein isoforms, low-molecular-weight GTPases, mitogen-activated protein kinase cascade, protein kinase C, calcineurin, Janus kinase, and type 2 (Th2) helper T cells, which regulate cell-mediated immunity and antibody-mediated humoral immune responses, respectively. STAT6-deficient (STAT6−/−) mice lost IL-4–mediated immune functions, including Th2 differ-

Received June 21, 2004; revision received August 18, 2004; accepted August 24, 2004.
From the Department of Internal Medicine and Therapeutics (S.H., O.Y., Y.H., S.H., T.T., K.K., T.W., M.T., M.A., K.N., M.H., K.O.), Graduate School of Medicine; First Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry (I.T.); Department of Medical Information Science (Y.M.), Graduate School of Medicine; and Department of Host Defense (S.A.), Research Institute for Microbial Disease, Osaka University, Suita, Osaka; and Department of Internal Medicine II, Graduate School of Medicine, Chiba University, Chiba (H.N.), Japan.
Correspondence to Kinya Otsu, MD, PhD, Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail kotsu@medone.med.osaka-u.ac.jp © 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000146798.70980.9A

2631
entiation, but developed a Th1 response with higher levels of IL-12, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).\textsuperscript{8} TNF-α was found to be induced in hearts with chronic heart failure and to have a direct detrimental effect on the myocardium.\textsuperscript{9,10} Transgenic mice with targeted overexpression of TNF-α exhibited concentric hypertrophy, heart failure, myocardial fibrosis, and cardiomyocyte apoptosis.\textsuperscript{11,12} Recent work on tristetraprolin (TTP), a zinc finger protein, has provided a new perspective on the regulation of TNF-α biosynthesis.\textsuperscript{13} TTP binds to the AU-rich element in the 3′ untranslated region of TNF-α mRNA to destabilize the mRNA. STAT6 signaling has been reported to induce TTP in mast cells, which then reduces the expression of TNF-α.\textsuperscript{14}

We therefore hypothesized that STAT6 is involved in cardiac remodeling mediated through TTP-regulated TNF-α expression.

In this report, we attempted to clarify the in vivo role of STAT6 using STAT6\textsuperscript{−/−} mice. The results indicate that STAT6 plays a protective role in response to pressure overload.

**Methods**

**Animals and In Vivo Studies**

This study was performed under the supervision of the Animal Research Committee in accordance with the guidelines on animal experiments of Osaka University and the Japanese government Animal Protection and Management Law (No. 105). Ten- to 12-week-old STAT6\textsuperscript{−/−} mice with a C57Bl6/J background\textsuperscript{8} and age-matched wild-type (WT) C57Bl6/J mice as controls were used in this study. Measurements of hemodynamic parameters were performed on mice anesthetized with intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (3 to 6 mg/kg) by using a 1.4F catheter (Miller).\textsuperscript{15} Echocardiography\textsuperscript{16} and thoracic transverse aortic constriction operation (TAC)\textsuperscript{15} were performed on mice anesthetized with intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (3 to 6 mg/kg), respectively.

**Western Blot Analysis**

Hearts were homogenized with a lysis buffer containing 40 mM/L Tris, 120 mM/L NaCl, 0.1% Nonidet P-40, 20 mM/L Na\textsubscript{2}VO\textsubscript{4}, 1 mM/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mL/L dithiothreitol. Denatured (95°C for 5 minutes for STAT6 and 22°C for 30 minutes for TTP) total protein homogenates (20 to 50 μg per lane) were subjected to Western blot analysis with the use of the antibody against phospho-STAT6 (Cell Signaling Technology), STAT6 or TTP (Santa Cruz Biotechnology), and 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 1 mmol/L dithiothreitol. Denatured (95°C for 5 minutes for STAT6 and 22°C for 30 minutes for TTP) total protein homogenates (20 to 50 μg per lane) were subjected to Western blot analysis with the use of the antibody against phospho-STAT6 (Cell Signaling Technology), STAT6 or TTP (Santa Cruz Biotechnology), and the ECL advance kit (Amersham).

**Evaluation of Fibrosis and Apoptosis**

The area of fibrotic lesion of myocardium was determined on computer-aided manipulator program Macscope (Mitani Corporation). To detect apoptotic cells, the terminal deoxynucleotidyl transferase–mediated biotin dUTP nick-end labeling (TUNEL) assay was performed with the use of the antibody against phospho-STAT6 (Cell Signaling Technology), STAT6 or TTP (Santa Cruz Biotechnology), and anti–α-sarcomeric actin antibody (Santa Cruz Biotechnology) as a primary antibody and biotinylated anti-goat immunoglobulin antibody as a secondary antibody. Sections were counterstained with methyl green.

**TUNEL and anti–α-sarcomeric actin antibody (Santa Cruz Biotechnology) as a primary antibody and biotinylated anti-goat immunoglobulin antibody as a secondary antibody. Sections were counterstained with methyl green.**

**RNA Dot Blot Analysis**

Total RNA was isolated from the ventricular apexes to be analyzed with the use of TRizol reagent (Life Technologies Inc). Quantitative assessment of atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), and α-skeletal actin (α-SKA) was performed by RNA dot blot analysis, as previously described.\textsuperscript{15}

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

The mRNA levels for cytokines were determined by quantitative real-time reverse transcriptase–polymerase chain reaction (QRT-PCR). Reverse transcription and amplification were performed with the use of the TaqMan Reverse Transcription Reagent (PerkinElmer Life Sciences) and Platinum Quantitative PCR SuperMix-UDG (Invitrogen Life Technologies), respectively. The PCR primer and probe sequences were as follows: TNF-α forward 5′-TCA CAGG A-CTCA ATGGGC CT TCTTC-3′, reverse 5′-CCTT GT GCAG A-AC TCA GG A-AGT GG-3′, probe 5′-AAT TCTAC GAG C-GCT CT GC-3′; IL-4 forward 5′-GCT CTA A CCCC CAG CT A- GTT T-3′, reverse 5′-GAT TCTG CTC GTG AT CATT C- G-3′, probe 5′-CAT CTC C T CTTCC TT-3′; IFN-γ forward 5′-CTGCT GT ATGG GAG AGATA GTGC T-3′, reverse 5′-TGCT GTG C TGC GCT GT A TTA-3′, probe 5′-GAC CAG C- GC-3′; IL-2 forward 5′-GCT AGT C A CTT CGCA C- TCCT T-3′, reverse 5′-GGGTG CGCT CTTG AC AA-3′, probe 5′-CAT TGA C A CTTG TC AT C-3′; IL-12 forward 5′-AGCT CGC A GCAA A -GCAA G-3′, reverse 5′-GCAA A A CAC GTTG C ACAA C-3′, probe 5′-CCT CAG A AGCT A AA C- A C T A-3′. Resulting PCR products were measured and elaborated by the sequence detector ABI7700 (PerkinElmer Biosynthesis). QRT-PCR standard curves were constructed with the use of mouse total RNA from splenocytes stimulated by lipopolysaccharide or antibodies against CD3 and CD28. All data were normalized to GAPDH content and expressed as fold increase over the sham-operated group.

**TTP Promoter Assay**

Mouse ventricular cardiomyocytes from 1- to 2-day-old WT and STAT6\textsuperscript{−/−} mice were prepared for luciferase assay.\textsuperscript{17} Luciferase reporter construct possessing murine TTP promoter (−691 to +59; TTP-691) and WT STAT6 expression vector (pcDNA3STAT6) were described previously.\textsuperscript{14} Mouse ventricular cardiomyocytes were transfected with 1.0 μg of TTP-691 along with 0.5 μg of pcDNA3STAT6 or pcDNA3 with the use of Lipofectin reagent (Life Technologies Inc).\textsuperscript{17} Cardiomyocytes were cultured in the presence of 100 μmol/L of endothelin-1 for 24 hours and subjected to luciferase assay with the use of a luciferase reporter assay kit (Promega). Luciferase activity was normalized to total protein concentration and expressed as n-fold stimulation relative to control.

**Statistical Analysis**

Results are shown as mean±SEM. Paired data were evaluated by Student t test. A 1-way ANOVA with the Bonferroni post hoc test or repeated-measures ANOVA was used for multiple comparisons. In QRT-PCR, statistical analysis was performed only when fold increase reached 2-fold. A value of \(P<0.05\) was considered statistically significant.
Physiological Parameters of STAT6−/− Mice at Basal Level

<table>
<thead>
<tr>
<th>Parameter</th>
<th>STAT6−/− (n=9)</th>
<th>WT (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>26.0±0.5</td>
<td>26.2±0.8</td>
<td>0.80</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>123.1±4.9</td>
<td>120.2±4.8</td>
<td>0.68</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>86.8±4.0</td>
<td>84.4±3.3</td>
<td>0.65</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>16.64±0.15</td>
<td>16.85±0.20</td>
<td>0.43</td>
</tr>
<tr>
<td>LV/body weight, mg/g</td>
<td>3.33±0.08</td>
<td>3.21±0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>LV/tibia length, mm</td>
<td>5.21±0.22</td>
<td>5.00±0.15</td>
<td>0.42</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.77±0.06</td>
<td>3.91±0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>2.25±0.05</td>
<td>2.35±0.06</td>
<td>0.18</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>81.0±3.5</td>
<td>79.1±1.8</td>
<td>0.63</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>3.8±1.0</td>
<td>2.0±0.8</td>
<td>0.17</td>
</tr>
<tr>
<td>dP/dtmax, mm Hg/s</td>
<td>5589±237</td>
<td>5910±356</td>
<td>0.47</td>
</tr>
<tr>
<td>dP/dtmin, mm Hg/s</td>
<td>−4011±231</td>
<td>−4520±242</td>
<td>0.15</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>373±13</td>
<td>402±17</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Results

Characterization of STAT6-Knockout Mice

There were no significant differences in weight of body, whole heart, or left ventricle (LV) between STAT6−/− and WT mice (Table). The STAT6−/− hearts showed no evidence of any cardiac morphological defects, nor did histological examination of the hearts demonstrate any myofibrillar disarray, necrosis, or ventricular fibrosis (data not shown). The echocardiographic parameters such as end-diastolic left ventricular internal dimension (LVIDd), end-systolic left ventricular internal dimension (LVIDs), fractional shortening (FS), septal wall thickness, and posterior wall thickness of STAT6−/− and WT mice were not significantly different (Table). Furthermore, hemodynamic data did not indicate any differences in LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), the maximum first derivative of LV pressure (LV dP/dtmax), the minimum first derivative of LV pressure (LV dP/dtmin), and heart rate between STAT6−/− and WT mice (Table). These findings indicate that the STAT6−/− mice had normal global cardiac structure and function.

Activation of STAT6 During Biomechanical Stress Induced by Pressure Overload

Next, STAT6−/− and WT mice were subjected to TAC, followed by evaluation of the activities of STAT6 in hearts. On activation, STAT6 is known to be tyrosine phosphorylated and translocated to nuclei.3 One week after TAC, tyrosine phosphorylation and translocation to nuclei of STAT6 had significantly increased in WT compared with sham-operated mice (Figure 1A, 1B, 1C). Immunolabeling of STAT6 was observed mainly in cardiomyocytes (Figure 1C). These results demonstrate that STAT6 was activated in response to pressure overload. There were no compensatory changes in the expression levels of STAT1, STAT2, or STAT3, which has been reported to be activated in response to pressure overload,18 in STAT6−/− mice both at baseline and 1 week after TAC compared with WT mice (data not shown).

Cardiac Function and Hypertrophy After TAC

In WT mice, the LVIDd and FS 1 week after TAC did not significantly differ from those of the sham-operated group. In contrast, LVIDd and LVIDs in STAT6−/− mice had significantly increased and FS was significantly reduced after TAC compared with corresponding values for the sham-operated group (Table). Furthermore, hemodynamic data did not indicate any differences in LV systolic pressure (LV dP/dtmax), the minimum first derivative of LV pressure (LV dP/dtmin), and heart rate between STAT6−/− and WT mice (Table). These findings indicate that the STAT6−/− mice had normal global cardiac structure and function.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>STAT6−/− (n=9)</th>
<th>WT (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>26.0±0.5</td>
<td>26.2±0.8</td>
<td>0.80</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>123.1±4.9</td>
<td>120.2±4.8</td>
<td>0.68</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>86.8±4.0</td>
<td>84.4±3.3</td>
<td>0.65</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>16.64±0.15</td>
<td>16.85±0.20</td>
<td>0.43</td>
</tr>
<tr>
<td>LV/body weight, mg/g</td>
<td>3.33±0.08</td>
<td>3.21±0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>LV/tibia length, mm</td>
<td>5.21±0.22</td>
<td>5.00±0.15</td>
<td>0.42</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.77±0.06</td>
<td>3.91±0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>2.25±0.05</td>
<td>2.35±0.06</td>
<td>0.18</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>81.0±3.5</td>
<td>79.1±1.8</td>
<td>0.63</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>3.8±1.0</td>
<td>2.0±0.8</td>
<td>0.17</td>
</tr>
<tr>
<td>dP/dtmax, mm Hg/s</td>
<td>5589±237</td>
<td>5910±356</td>
<td>0.47</td>
</tr>
<tr>
<td>dP/dtmin, mm Hg/s</td>
<td>−4011±231</td>
<td>−4520±242</td>
<td>0.15</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>373±13</td>
<td>402±17</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Figure 1. Activation of STAT6 in response to pressure overload. A, Homogenates of hearts obtained from WT and STAT6−/− mice 1 week after TAC were subjected to Western blot analysis with anti-phospho-STAT6 and anti-STAT6 antibodies. B, Densitometric analysis. Phospho-STAT6/total STAT6 in a sham-operated WT is expressed as 1. Bars represent mean±SEM values (sham group, n=3; TAC group, n=4). *P<0.05 vs sham-operated WT control. C, Translocation of STAT6 to nuclei in response to pressure overload. Triple staining (anti-STAT6 antibody, anti-α-sarcomeric actin antibody, and propidium iodide) was performed on sham-operated WT, TAC-operated WT, and sham-operated STAT6−/− mice 1 week after operation. Staining for propidium iodide or anti-α-sarcomeric actin antibody is shown in red, and that for STAT6 is shown in green. In the overlay image, nuclei to which STAT6 translocates are shown in yellow (white arrows).
group (Figure 2A, 2B, 2C, 2D). Furthermore, although the lung/body weight ratio, the index of lung congestion, did not differ significantly between sham- and TAC-operated groups in WT mice, TAC caused a significant increase in this parameter in STAT6/−/− mice compared with sham-operated STAT6/−/− mice (Figure 2E). These findings indicate that STAT6/−/− mice developed congestive heart failure after TAC.

We also examined pressure overload–induced hypertrophic responses in STAT6/−/− mice. TAC induced significant increases in LV/body weight ratio and LV weight/tibia length ratio in STAT6/−/− compared with sham-operated STAT6/−/− mice (Figure 3B). The myocyte cross-sectional area was measured by tracing the outline of 100 to 200 myocytes in each section. *P<0.05 vs corresponding sham-operated mice. D, mRNA expression evaluated by dot blot analysis.

hypertrophic parameters were not significantly different between TAC-operated STAT6/−/− and WT mice.

**Histological Examination**

In comparison with those of sham-operated mice, hematoxylin-eosin–stained sections of both WT and STAT6/−/− mice after TAC showed significant LV hypertrophy, but LV chamber enlargement was seen only in STAT6/−/− mice after TAC (Figure 4A, 4B). Masson-trichrome staining demonstrated that interstitial fibrosis was present in both WT and STAT6/−/− mice after TAC, but the extent of fibrosis was significantly higher in STAT6/−/− mice compared with that in WT mice (21.5±1.2% for STAT6/−/−, 11.9±1.5% for WT; P<0.05) (Figure 4A, 4B).

**Cardiomyocyte Apoptosis in Response to Pressure Overload**

TUNEL assay was performed to examine whether apoptosis is involved in pressure overload–induced heart failure in STAT6/−/− mice. TUNEL-positive cells contained condensed

![Figure 2](image2.png)

**Figure 2.** Morphological and functional results of pressure overload in STAT6/−/− hearts. A, M-mode echocardiographic tracings from WT and STAT6/−/− mice before and 1 week after TAC. B, FS. C, LVIDd. D, LVIDs. Sham-operated WT (n=5), TAC-operated WT (n=15), sham-operated STAT6/−/− (n=5), and TAC-operated STAT6/−/− (n=13) mice are shown. E, Lung weight/body weight 1 week after TAC or sham operation. Sham-operated WT (n=5), TAC-operated WT (n=11), sham-operated STAT6/−/− (n=5), and TAC-operated STAT6/−/− (n=12) mice are shown. *P<0.05 vs TAC-operated WT; †P<0.05 vs corresponding sham-operated controls.

![Figure 3](image3.png)

**Figure 3.** Cardiac hypertrophic responses to pressure overload. LV weight/body weight (LVW/BW) (A) or LVW/tibia length (B) was obtained 1 week after TAC. Sham-operated WT (n=4), TAC-operated WT (n=8), sham-operated STAT6/−/− (n=5), and TAC-operated STAT6/−/− (n=10) mice are shown. C, Myocyte cross-sectional area was measured by tracing the outline of 100 to 200 myocytes in each section. *P<0.05 vs corresponding sham-operated mice. D, mRNA expression evaluated by dot blot analysis.
chromatin, a characteristic of apoptosis (Figure 4C), and were identified as cardiomyocytes by anti–α-sarcomeric actin staining. The number of TUNEL-positive cells had significantly increased 2 days and 1 week after TAC in both STAT6−/− and WT hearts compared with that in the sham-operated groups (Figure 4D, 4E). However, the number of TUNEL-positive cells was significantly higher in STAT6−/− mice than in WT mice 2 days and 1 week after TAC.

**Pressure Overload–Induced Cytokine Expression**

We examined how the expression of cytokines was affected by pressure overload in STAT6−/− mice. QRT-PCR analyses showed that mRNA expression of TNF-α after TAC, expressed as the expression level relative to that in sham-operated mice, had increased in both STAT6−/− and WT mice 12 hours after TAC. Although the TNF-α mRNA level in WT returned to baseline level within 24 hours after TAC, that in STAT6−/− mice remained higher than that in WT mice until 2 days postoperatively (Figure 5A). We also examined the mRNA expression levels of other cytokines such as IL-2, IL-4, IL-12, and IFN-γ and found that the expression level of IL-4 or IFN-γ had not changed significantly after TAC (Figure 5B, 5C) and that no expression of IL-2 or IL-12 was detected in the hearts (data not shown). Immunohistochemical analysis with the use of anti–TNF-α antibody revealed that STAT6−/− hearts showed enhanced expression of TNF-α compared with that in WT hearts 2 days after TAC, in agreement with the results of QRT-PCR (Figure 5D, 5E). Although TNF-α was detected in noncardiomyocytes including interstitial cells and endothelial cells, most of the TNF-α-expressing cells were cardiomyocytes.

In regard to the mechanism of upregulation of TNF-α in STAT6−/− mice, STAT6 reduces the expression of TNF-α by induction of TTP in mast cells. When we determined TTP protein expression levels 24 hours after TAC, the point at which the TNF-α mRNA level was significantly higher in STAT6−/− than in WT mice, TTP protein expression in WT mice had significantly increased compared with that in sham-operated mice (Figure 6A). The TTP level in STAT6−/− mice, on the other hand, had not increased after TAC (Figure 6A). The TTP level showed no difference between sham-operated WT and STAT6−/− mice.

To determine whether induction of TTP after TAC depends on STAT6 signaling, we performed a TTP reporter assay using neonatal cardiomyocytes isolated from STAT6−/− and WT mice. TTP-691Luc, a reporter construct in which the TTP promoter drives the luciferase gene, was significantly activated by 100 nmol/L of endothelin-1 in WT but not in STAT6−/− cardiomyocytes (Figure 6B). The control luciferase activities in WT and STAT6−/− cardiomyocytes showed no difference. The lack of activation by endothelin-1 in hearts 2 days after TAC. In the overlay image, a nucleus stained by both TUNEL and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow. D, WT and propidium iodide is shown in yellow.
STAT6−/− cardiomyocytes was compensated by forced expression of STAT6. These results indicate that TTP was induced through the STAT6 signaling pathway.

Discussion
The results of this study, in which we examined an in vivo role of STAT6 in the heart using STAT6−/− mice, show for the first time that STAT6 plays a protective role against hemodynamic overloading. STAT6−/− mice exhibited normal global cardiac structure and function, indicating that there is no cardiomyocyte cell-autonomous requirement for the STAT6 signaling pathway during normal embryonic development. Furthermore, the STAT6 pathway also does not appear to be required for normal heart growth in the postnatal period. STAT6 is activated in neonatal cardiomyocytes treated with angiotensin II and in the hypertrophied hearts of the spontaneously hypertensive rat.5 In this study we showed that STAT6 is activated in mouse hearts after TAC. This suggests the involvement of STAT6 in cardiac hypertrophy. However, the hypertrophic responses after TAC showed no significant differences between STAT6−/− mice and controls, indicating that STAT6 does not play an essential role in the development of cardiac hypertrophy induced by pressure overload.

In response to biomechanical stress such as pressure or volume overload, the heart activates an adaptive physiological response in the form of cardiac hypertrophy, whereas long-lasting or excessive exposure to mechanical stress results in transition to heart failure. In our study STAT6 was activated after TAC in WT mouse hearts, whereas STAT6−/− mice were unable to maintain LV function. The increased apoptotic response to pressure overload indicates that the decreased LV function in STAT6−/− hearts is due to myocardial cell loss accompanied by fibrosis. However, we cannot exclude the possibility that the loss of contractility of individual existing cardiomyocytes that lack STAT6 contributes to the impaired LV function, taking into consideration that TNF-α, a possible downstream target of STAT6, depresses contractile function of the hearts.

In STAT6−/− mice, the production of Th2 cytokines such as IL-4, IL-5, and IL-10 was completely inhibited in lymph nodes, whereas that of Th1 cytokines such as TNF-α and IFN-γ was higher than in controls.8 We found no significant differences in the level of IFN-γ or IL-4 between STAT6−/− hearts and controls. Moreover, any TAC-induced changes in Th1 or Th2 cytokine expression level were not detected in lymph nodes in STAT6−/− or WT mice (data not shown). These data indicate that a defect in the Th2 responses in STAT6−/− mice is not responsible for the observed phenotypes. We observed prolonged induction of TNF-α mRNA in STAT6−/− hearts but only transient induction in controls. Thus, prolonged TNF-α mRNA, but not the early transient induction, appears to be STAT6 dependent. Transgenic mice with targeted overexpression of TNF-α exhibited heart failure with myocardial fibrosis.11,12 suggesting that sustained induction of TNF-α might play a significant role in the development and progression of chronic heart failure. One week after TAC, WT developed cardiac hypertrophy without any sign of heart failure. Prolonged, but not transient, TNF-α expression may have a deleterious effects on LV function, although the possibility cannot be excluded that factors other than TNF-α may cause the observed phenotypes in STAT6−/− mice. Furthermore, the significance of the transient induction of TNF-α observed in WT mice remains to be elucidated. We have previously reported that a bolus injection of TNF-α induces cardiac protection against lethal ischemia.19 The transient induction of TNF-α might be involved in stress adaptation in response to pressure overload.

The findings of our study confirm those of a previous study that TAC provoked an increase in the expression of TTP with a transient increase in TNF-α mRNA.20 This suggests that TTP is responsible for the decrease in TNF after its transient increase after TAC. On the other hand, we observed a more extended increase in TNF-α mRNA in STAT6−/− hearts. It has been reported that STAT6 induces TTP expression.14 It has also been reported that G-protein–coupled receptor agonists such as angiotensin II activate STAT6 in isolated cardiomyocytes.5 In our study we observed no increase in TTP protein in STAT6−/− mouse hearts after TAC, and we observed that a G-protein–coupled receptor agonist activated TTP promoter activity in a STAT6-dependent manner. Taken together, the prolonged increase in TNF-α mRNA appears to be due to a lack of STAT6-dependent TTP induction in response to pressure over-
load in STAT6−/− hearts. As a limitation of the study, the study did not confirm whether prolonged expression of TNF-α is caused by the loss of TTP induction and whether such expression is responsible for the observed cell loss and impaired LV function. Further study will be necessary to elucidate roles of TNF-α and TTP in the pathogenesis of the impaired LV function observed in STAT6−/− mice.

In conclusion, the ablation of STAT6 resulted in an inability to maintain LV function due to an apoptotic response to pressure overload. This implies a protective function of STAT6 against stress in hearts. STAT6 may constitute a molecular target for the prevention and treatment of heart failure.

References

Pressure Overload Induces Cardiac Dysfunction and Dilation in Signal Transducer and Activator of Transcription 6–Deficient Mice
Shungo Hikoso, Osamu Yamaguchi, Yoshiharu Higuchi, Shinichi Hirotani, Toshihiro Takeda, Kazunori Kashiwase, Tetsuya Watanabe, Masayuki Taniike, Ikuko Tsujimoto, Michio Asahi, Yasushi Matsumura, Kazuhiko Nishida, Hiroshi Nakajima, Shizuo Akira, Masatsugu Hori and Kinya Otsu

_Circulation_. 2004;110:2631-2637; originally published online October 18, 2004; doi: 10.1161/01.CIR.0000146798.70980.9A
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/17/2631

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

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

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