Blockade of MyD88 attenuates cardiac hypertrophy and decreases cardiac myocyte apoptosis in pressure overload-induced cardiac hypertrophy in vivo

Tuanzhu Ha,1 Fang Hua,1 Yuehua Li,1 Jing Ma,1 Xiang Gao,2 Jim Kelley,3 Aiqiu Zhao,4 Georges E. Haddad,5 David L. Williams,1 I. William Browder,1 Race L. Kao,1 and Chuanfu Li1

Departments of 1Surgery and 3Internal Medicine, East Tennessee State University, Johnson City, Tennessee; 2Animal Model Research Center, Nanjing University, Nanjing, China; and Department of 4Physiology and Biophysics, Howard University, Washington DC

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Ha, Tuanzhu, Fang Hua, Yuehua Li, Jing Ma, Xiang Gao, Jim Kelley, Aiqiu Zhao, Georges E. Haddad, David L. Williams, I. William Browder, Race L. Kao, and Chuanfu Li. Blockade of MyD88 attenuates cardiac hypertrophy and decreases cardiac myocyte apoptosis in pressure overload-induced cardiac hypertrophy in vivo. Am J Physiol Heart Circ Physiol 290: H985–H994, 2006. First published September 30, 2005; doi:10.1152/ajpheart.00720.2005.—In this study, we evaluated whether blocking myeloid differentiation factor-88 (MyD88) could decrease cardiac myocyte apoptosis following pressure overload. Adenovirus expressing dominant negative MyD88 (Ad5-dnMyD88) or Ad5-green fluorescent protein (GFP) (Ad5-GFP) was transfected into rat hearts (n = 8/group) immediately followed by aortic banding for 3 wk. One group of rats (n = 8) was subjected to aortic banding for 3 wk without transfection. Sham surgical operation (n = 8) served as control. The ratios of heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL) were calculated. Cardiomyocyte size was examined by FITC-labeled wheat germ agglutinin staining of membranes. Cardiac myocyte apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay, and myocardial interstitial fibrosis was examined by Masson’s Trichrome staining. Aortic banding significantly increased the HW/BW by 41.0% (0.44 ± 0.03 vs. 0.31 ± 0.008), HW/TL by 47.2% (42.7 ± 1.30 vs. 29.0 ± 0.69), cardiomyocyte size by 49.6%, and cardiac myocyte apoptosis by 11.5%, and myocardial fibrosis and decreased cardiac function compared with sham controls. Transfection of Ad5-dnMyD88 significantly reduced the HW/BW by 18.2% (0.36 ± 0.006 vs. 0.44 ± 0.013) and HW/TL by 22.3% (33.2 ± 0.95 vs. 42.7 ± 1.30) and decreased cardiomyocyte size by 56.8%, cardiac myocyte apoptosis by 76.2%, as well as fibrosis, and improved cardiac function compared with aortic-banded group. Our results suggest that MyD88 is an important component in the Toll-like receptor-4-mediated nuclear factor-κB activation pathway that contributes to the development of cardiac hypertrophy. Blockade of MyD88 significantly reduced cardiac hypertrophy, cardiac myocyte apoptosis, and improved cardiac function in vivo.

IN VITRO STUDIES show that nuclear factor-κB (NF-κB) activation is involved in the hypertrophic response of cultured cardiomyocytes (4, 8, 12, 14, 27, 32). Inhibition of NF-κB activation significantly attenuates hypertrophic response in cultured cardiomyocytes (8, 27). We have demonstrated that NF-κB activation is required for the development of cardiac hypertrophy in vivo (21). NF-κB activation has been shown to play a critical role in regulating expression of groups of genes involved in immune and inflammatory responses, cell death and survival, cell growth, and cell cycle (2). NF-κB is a transcription factor in Toll-like receptor(s) (TLRs)-mediated signaling pathways (1, 23, 34). The main pathway of TLR-mediated signaling that leads to NF-κB activation involves the adaptor molecule termed myeloid differentiation factor-88 (MyD88), kinases of the IL-1 receptor-associated kinase (IRAK) family, TNF-receptor-associated factor 6 (TRAF6), NF-κB-inducing kinase (NIK), transforming growth factor (TGF)-β-activated kinase 1 (TAK1), and 1κB kinase (IKK) complexes (1, 34). MyD88 has been demonstrated to play an important role in the TLR-interleukin (IL)-receptor (TIR)-mediated NF-κB activation pathway (23). We have recently observed that TLR4 is an important receptor contributing to cardiac hypertrophy in vivo (13). MyD88 is a key adaptor protein for TLR signaling. Blockade of MyD88-mediated signaling attenuated the development of cardiac hypertrophy in vivo (22). It has been shown that cardiac myocyte apoptosis plays an important role in the transition of hypertrophic heart to heart failure (9, 25, 29). In the present study we examined whether blocking MyD88 could prevent cardiac myocyte death and improve cardiac function following aortic banding in vivo. We have observed that transfection of dominant negative MyD88 into the myocardium significantly reduced cardiac myocyte apoptosis and improved cardiac function in pressure overload-induced cardiac hypertrophy in rats. The results suggest that the MyD88-dependent signaling pathway plays a role in the cardiac myocyte death in pressure overload-induced cardiac hypertrophy in vivo.

EXPERIMENTAL PROCEDURES

Aortic banding induced cardiac hypertrophy in rats. Male Sprague-Dawley rats (225–250 g) were maintained in the Division of Laboratory Animal Resources at East Tennessee State University (ETSU) in accordance with the guidelines for the “Principles of Laboratory Animal Care” and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All aspects of the animal care and experimental protocols were approved by the ETSU Committee on Animal Care. Cardiac hypertrophy in rats was induced as previously described (21). Briefly, rats were anesthetized with isoflurane inhalation and ventilated with room air with the use of a rodent ventilator. The hearts were exposed through a left thoracotomy in the fourth intercostal space, and a suture (2–0 silk) was drawn under the ascending aorta. An 18-gauge needle was placed alongside the ascending
aorta, and the suture was tightly tied together with the needle. The needle was then removed and the thorax was closed. The pressure gradient of aortic coarctation was 63 ± 2.5 mmHg after aortic banding. For the sham control, the identical procedure was performed, except without the tying of the suture around the aorta. Three weeks after the operation, the heart weight-to-body weight (HW/BW) ratio was calculated.

**Construction of adenovirus expressing dominant negative MyD88.** The dominant negative MyD88 (dnMyD88) DNA was kindly provided by Dr. Jurg Tschopp at the Institute of Biochemistry, University Lausanne, Switzerland (6, 15). This dnMyD88 construct has been shown to significantly inhibit IL-1-induced NF-κB activation (6). To construct adenovirus expressing dnMyD88 (Ad5-dnMyD88), PCR was performed to amplify the dnMyD88 fragments containing FLAG (an eight amino acid sequence tag, Sigma) from the pCR3.V64-Met-Flag-Stop vector. The amplified DNA fragments were inserted into pShuttle vector at Hoxl and KpnI sites followed by recombinant with double-deleted adenovirus type 5 (Ad5) vector. The double-deleted adenovirus expressing dnMyD88 (Ad5-dnMyD88) was packaged in 293A cells and purified by ultracentrifugation (21). The plaque forming units per milliliter (pfu/ml) of Ad5-dnMyD88 were determined by tissue culture infectious dose 50 (TCID50) method according to the manufacturer’s protocols (Quantum Biotechnologies, Montreal, Canada).

**Expression of Ad5-dnMyD88 in H9C2 cardiomyoblasts.** H9C2 rat cardiomyoblast cell line was obtained from the American Type Culture Collection (Rockville, MD) and was maintained in Dulbecco’s modified Eagle’s medium (DMEM)-supplemented 10% fetal bovine serum (FBS) under 5% CO2 at 37°C. When the cells reached 70–80% confluence, Ad5-dnMyD88 or Ad5-GFP (1 × 1010 pfu/ml, pfu) was added into the cells. Twenty-four hours after transfection, the cells were stimulated with lipopolysaccharide (LPS) at 0.5 μg/ml for 30 min. The cells were harvested for analysis of NF-κB binding activity by EMSA (17–20) and for the expression of the transfected dnMyD88 and FLAG by Western blot analysis.

**Adenovirus-mediated gene transfection into the myocardium in vivo.** Transfection of Ad5-dnMyD88 (1 × 1010 pfu/ml) into the myocardium was performed as previously described (21). Ad5-GFP (1 × 1010 pfu/ml) served as the control. The transfection efficiency was examined by evaluation of green fluorescence protein (GFP) expression in the hearts (n = 3) 3 days after transfection of Ad5-GFP (21). The expressions of transfected dnMyD88 and FLAG were examined by Western blot analysis with specific anti-MyD88 and FLAG antibodies in the hearts (n = 2) at 3 days and 1, 2, and 3 wk after transfection of Ad5-dnMyD88. To determine the effect of transfected dnMyD88 on cardiac hypertrophy, Ad5-dnMyD88 (1 × 1010 pfu/ml) was transfected into the myocardium (n = 8) immediately followed by banding the aorta. Transfection of Ad5-GFP served as a control (n = 8). Two rats from each group were subjected to measurement of the pressure gradients 3 days after aortic banding, and the pressure gradients were 59 and 63 mmHg in the aortic banding group, 60 and 64 mmHg in the GFP group, and 66 and 59 mmHg in the dnMyD88 group. Three weeks after aortic banding was completed, the hearts were harvested, and the HW/BW was calculated. We chose the 3-wk time point because we have previously shown that transfection of adenovirus expressing IkBα mutant into the myocardium had a significant effect on the attenuation of cardiac hypertrophy (21).

**Electrophoretic mobility shift assay.** NF-κB activity was examined by electrophoretic mobility shift assay (EMSA) as previously described (17–20) in a 15-μl binding reaction mixture containing 15 μg of nuclear proteins and 35 fmols of γ-32P-labeled double-stranded NF-κB consensus oligonucleotide. A supershift assay using antibodies to P65 and P50 was performed to confirm NF-κB binding specificity as previously described (17–20).

**Western blot analysis.** Western blot analysis was performed as previously described (17–20) with primary anti-FLAG and anti-MyD88 (Sigma), respectively, followed by peroxidase-conjugated second antibodies (Cell Signaling Technology). The membranes were analyzed by the ECL system (Amersham Pharmacia). The same membranes were probed with anti-GAPDH (Biodesign, Saco, ME) after being washed with stripping buffer. The signals were quantified by scanning densitometry and computer-assisted image analysis.

**Histology.** To evaluate the expression of GFP, the hearts were harvested, sectioned, and immersion-fixed in 4% buffered paraformaldehyde for 3 days after transfection of Ad5-GFP. The tissues were cut at 5 μm, counterstained with hematoxylin and eosin, and examined by fluorescence microscopy (21). To examine histological change in aortic banding-induced cardiac hypertrophy, hearts were harvested 3 wk after aortic banding with or without Ad5-dnMyD88 transfection (n = 4 hearts/group) and prepared in the standard manner as previously described (21). Myocardial interstitial fibrosis was stained with Masson’s Trichrome. The cardiac myocyte size was examined by FITC-labeled wheat germ agglutinin (Sigma) staining of membranes, and left ventricular cardiac myocyte membranes were observed by fluorescein microscopy. Morphometric analysis was performed with a pheno-Image system (Bio-Rad). A value from each heart was calculated by use of the measurements of 40–50 cells from an individual heart. The results were expressed as mean values ± SE from each experimental group.

**In situ apoptosis assay.** In situ cell death examination was performed as previously described (18). Hearts harvested from each group (n = 4/group) were sectioned and embedded in paraffin. Three slides from each block were evaluated for percentage of apoptotic cells by using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay (Boehringer Mannheim, Indianapolis, IN). Four slide fields were randomly examined by using a defined rectangular field area with magnification ×200. Fifty cells were counted in each field.

**Immunohistochemistry.** Caspase-3 activity was assessed in the heart sections by immunohistochemistry using specific anti-caspase-3 cleaved antibody (Cell Signaling Technology) (31). Briefly, hearts from each group (n = 4) were harvested and immersion fixed in 4% buffered paraformaldehyde, embedded in paraffin, cut at 5 μm, and stained with an antibody directed against activated caspase-3 (31). Three slides from each block were evaluated with brightfield microscopy.

**Hemodynamic measurements.** Rats were anesthetized with isoflurane inhalation and ventilated with room air with the use of a rodent ventilator. A microconductance pressure catheter (Millar Instruments, Houston, TX) was positioned in the left ventricle (LV) via the right carotid artery for continuous registration of LV pressure-volume loops (16) by using the PowerLab system (AD Instruments, Colorado Springs, CO). A cuvette calibration method was used to convert the conductance voltage into volume units by filling nonconductive cuvettes of known diameter with heparin-treated rat blood. Parallel conductance from surrounding structures was determined by intravenous (external jugular vein) injection of a small bolus (15 μl) of hypertonic saline (15% NaCl). All measurements were performed while ventilation was turned off momentarily. Indexes of systolic and diastolic cardiac performance were derived from LV pressure-volume data obtained at steady state. Cardiac output, ejection fraction, stroke volume, and stroke work were chosen as indexes for cardiac function change.

**Statistical analysis.** Results are expressed as means ± SE. For tests of significance between the groups, one-way analysis of variance (ANOVA) and Tukey’s procedure for multiple range tests were performed. P < 0.05 was considered to be significant.

**RESULTS**

**Expression of dnMyD88 in H9C2 cardiomyoblasts.** The expression of Ad5-dnMyD88 in the rat cardiomyoblasts cell line H9C2 (n = 3) was examined 24 h after transfection with
Ad5-dnMyD88 (1 × 10^7 pfu/ml). Ad5-GFP served as a control (n = 3). Because Ad5-dnMyD88 contains a FLAG epitope, we also examined the expression of FLAG with a specific antibody. As shown in Fig. 1, dnMyD88 and FLAG migrated with a molecular mass of 17 kDa in SDS-PAGE (6) and were detected in the cells transfected with Ad5-dnMyD88 but not detected in untransfected cells or in cells transfected with Ad5-GFP. 

Transfection of dnMyD88 inhibits LPS-induced NF-κB activation in H9C2 cardiomyoblasts. To examine the effect of Ad5-dnMyD88 on NF-κB activation, rat cardiomyoblasts were transfected with Ad5-dnMyD88 (n = 3) (1 × 10^7 pfu/ml), and Ad5-GFP served as a control (n = 3). Twenty-four hours after transfection, the cells were stimulated with LPS (0.5 μg/ml) for 30 min before NF-κB binding activity was examined by EMSA as previously described (17–20). As shown in Fig. 2, transfection of Ad5-dnMyD88 into H9C2 cells significantly inhibited NF-κB activation stimulated by LPS. Transfection of Ad5-GFP did not affect LPS-induced NF-κB activation.

Expression of dnMyD88 in the myocardium. The transfection efficiency and the expression of Ad5-dnMyD88 in the myocardium were evaluated in the hearts 3 days after transfection with Ad5-GFP or Ad5-dnMyD88 at 1 × 10^10 pfu/ml. GFP expression was examined by fluorescence microscopy, and dnMyD88 expression was examined by Western blot analysis with anti-MyD88 antibody. We also examined the expression of FLAG epitope by Western blot analysis with specific antibody. As shown in Fig. 3A, GFP expression was observed in most cardiac cells, including cardiomyocytes, after transfection of Ad5-GFP into the myocardium. Figure 3B shows that expressed dnMyD88 and FLAG were detected in the hearts 3 days and 1 and 2 wk after transfection with Ad5-dnMyD88 but not detected in the hearts transfected with Ad5-GFP. There was no detectable transfected dnMyD88 or FLAG 3 wk after transfection.

Transfection of dnMyD88 attenuates cardiac hypertrophy. To determine whether blocking MyD88-dependent NF-κB activation could attenuate the development of cardiac hypertrophy in vivo, we transfected Ad5-dnMyD88 (n = 8) or Ad5-GFP (n = 8) into the myocardium immediately followed by aortic banding. Three weeks after aortic banding, the hearts were harvested and HW/BW and heart weight/tibia length (HW/TL) were calculated. As shown in Fig. 4, A and B, aortic banding for 3 wk significantly increased HW/BW by 41.0% (0.44 ± 0.013 vs. 0.31 ± 0.008) and HW/TL by 47.2%
Transfection of Ad5-dnMyD88 significantly reduced HW/BW by 18.2% (0.36 ± 0.006 vs. 0.44 ± 0.013) and HW/TL by 22.3% (33.2 ± 0.95 vs. 42.7 ± 1.30), respectively, compared with aortic-banded hearts without transfection. Transfection of Ad5-GFP did not significantly alter the aortic banding-increased ratio of HW/BW. The reduced HW/BW by dnMyD88 was due to decreased left ventricular weight, and there were no differences in body weights compared with the untreated group (sham: 317.1 ± 7.36 g; banding: 315.0 ± 3.82 g; GFP: 318.3 ± 5.63 g; dnMyD88: 312.0 ± 4.89 g). Figure 4C shows that the cardiac myocyte area from the left ventricle of aortic-banded hearts was increased by 49.6% compared with sham control. The cell area of hearts from aortic-banded rats transfected with dnMyD88 was increased by 21.4% compared with the dnMyD88-transfected sham control. Thus transfection of dnMyD88 suppressed the aortic banding increase in cardiac myocyte size (Fig. 4C).

Transfection of dnMyD88 inhibits aortic banding-increased NF-κB activation. To investigate whether blocking MyD88-mediated signaling will decrease NF-κB activation in the hearts subjected to aortic banding, we transfected Ad5-dnMyD88 into the myocardium immediately followed by banding the aorta (n = 8). Transfection of Ad5-GFP served as control (n = 8). Three weeks after transfection, hearts were harvested and nuclear proteins were isolated for measurement of NF-κB binding activity by EMSA. As shown in Fig. 5A, aortic banding for 3 wk significantly increased NF-κB binding activity by 145.4% compared with sham controls. Transfection of Ad5-dnMyD88, however, significantly reduced NF-κB binding activity by 41.8% compared with the untreated aortic-banded group. Transfection of Ad5-GFP did not affect aortic banding-increased NF-κB binding activity in the myocardium. The specificity of NF-κB activity in the hypertrophic hearts was confirmed by the addition of 100-fold excess of unlabeled NF-κB or activated protein II oligonucleotides into the EMSA reaction. Unlabeled NF-κB oligonucleotides competed for the binding nuclear proteins prepared from aortic-banded rat hearts (Fig. 5B), whereas the activated protein II oligonucleotides did not. The predominant protein complex of NF-κB containing P50 and P65 subunits in the hypertrophic hearts was demonstrated by antibody supershift assays. Both antibodies, when added separately or together, shifted the major NF-κB binding complex.

Transfection of dnMyD88 reduces cardiac myocyte apoptosis following pressure overload. Aortic banding-induced cardiac hypertrophy exhibited extensive cardiac myocyte death and increase in fibrosis (Fig. 6, A–C). The TUNEL assay shows that cardiac myocyte apoptosis in aortic-banded hearts was significantly increased by 11.5% compared with sham control (Fig. 6B). Caspase-3 activity in the aortic-banded hearts was also increased as evidenced by immunohistochemistry with specific anti-cleaved caspase-3 antibody (Fig. 6C). Myocardial fibrosis was also increased in the hypertrophic hearts as evi-
Fig. 4. Transfection of Ad5-dnMyD88 attenuates aortic banding-induced cardiac hypertrophy. Ad5-dnMyD88 or Ad5-GFP (1 × 10^10 pfu/ml) was transfected into rat hearts immediately followed by banding the aorta. Three weeks after aortic banding, the hearts were harvested and the ratios of heart weight to body weight (HW/BW) (A) and heart weight to tibia length (HW/TL) (B) were analyzed. There were 8 rats per group. Photographs of representative hearts are shown at the top of A. C: transfection of Ad5-dnMyD88-reduced aortic banding increased cardiac myocyte size. The cardiac myocyte size was examined by FITC-labeled wheat germ agglutinin staining. Left ventricular cardiac myocyte membranes were observed by fluorescence microscopy. Representative heart sections (right) from sham, aortic-banded rats, aortic-banded rats transfected with Ad5-GFP or Ad5-dnMyD88. Left, quantitative analysis of cardiac myocyte size. *P < 0.05 compared with age-matched, sham-operated controls; #P < 0.05 compared with aortic banding group; &P < 0.05 compared with aortic banding + GFP group.
denced by Masson’s Trichrome staining (Fig. 6D). To evaluate whether blocking the MyD88-dependent NF-κB activation pathway could improve morphology of aortic-banded hearts, we transfected Ad5-dnMyD88 into the myocardium immediately followed by aortic banding for 3 wk. We observed that transfection of dominant negative MyD88 significantly improved the morphology of aortic-banded hearts (Fig. 6A). In Ad5-MyD88-transfected hearts, cardiac myocyte apoptosis was only increased 2.7% following aortic banding for 3 wk (Fig. 6B). Thus blocking the MyD88-dependent pathway reduced aortic banding-induced cardiac myocyte apoptosis by 76.2%. Caspase-3 activity was also reduced in the hearts transfected with Ad5-dnMyD88 (Fig. 6C). In addition, transfection of Ad5-dnMyD88 significantly reduced myocardial fibrosis in aortic-banded hearts (Fig. 6D). Transfection of Ad5-GFP had no effect on the improvement of morphology, cardiac myocyte apoptosis, and myocardial fibrosis in aortic-banded hearts (Fig. 6, A–C).

**Hemodynamic measurements.** In vivo cardiac function was measured 3 wk after aortic banding by the Millar pressure-volume conductance system. Figure 7A shows that aortic banding-induced cardiac hypertrophy resulted in significant reduction of cardiac output by 60.0%, ejection fraction by 53.6%, stroke volume by 58.0%, and stroke work by 47.7% compared with sham control. Blockade of MyD88 by transfection of Ad5-dnMyD88, however, significantly improved car-
DECREASED CARDIAC MYOCYTE DEATH BY BLOCKING MyD88

A

Sham

Banding

GFP

dnMyD88

4 x

10 x

20 x

20 μm

B

Sham

Banding

Control

GFP

dnMyD88

20 μm

C

Sham

Banding

Control

GFP

dnMyD88

20 μm

D

Sham

Banding

GFP

dnMyD88

10 x

20 x

20 μm
diac function, evidenced by increased cardiac output by 33.3%,
ejection fraction by 32.8%, stroke volume by 38.0%, and
stroke work by 31.1% when compared with aortic-banded rats.
Transfection with Ad5-GFP did not produce any significant
changes compared with control animals. Representative pres-
sure-volume loops from each group of animals are also pre-
sented (Fig. 7B)

**DISCUSSION**

A significant finding of this study is that blocking the
MyD88-mediated NF-κB activation pathway by transfection of
an adenovirus expressing dominant negative MyD88 (Ad5-
dnMyD88) into the myocardium significantly reduced cardiac
myocyte apoptosis and improved cardiac function following
pressure overload. This result suggests that the MyD88-depen-
dent NF-κB activation pathway contributes to cardiac myocyte
apoptosis in pressure overload-induced cardiac hypertrophy in
vivo.

MyD88 is an important immediate downstream adaptor
molecule that interacts directly with the TIR domain of TLRs
on the cell plasma membrane (23). After recognition of ligands
by TLRs, MyD88 recruits IRAK, which stimulates TRAF6,
leading to activation of IKKs, which stimulates IkB phosphorylation and degradation, resulting in NF-κB translocation
to the nucleus, binding to target DNA sequences, and stimu-
lation of gene expression. A role of NF-κB activation in the
hypertrophic response of cultured cardiomyocytes has been
documented. For example, activation of NF-κB is required for
hypertrophic growth of cultured neonatal cardiomyocytes (27)
and for myotrophin-induced cardiac hypertrophy in vitro (12).
A20, a feedback inhibitor of NF-κB activation (8), attenuated
the hypertrophic response of cardiomyocytes in vitro through
the inhibition of NF-κB signaling (8). We have previously demonstrated that NF-κB activation is required for the development of cardiac hypertrophy in vivo and inhibiting NF-κB activation attenuated aortic banding-induced cardiac hypertrophy (21). We have also observed that TLR4-deficient mice are resistant to aortic banding-induced cardiac hypertrophy (13), suggesting that TLR4 could be an important contributor to cardiac hypertrophy in vivo. Collectively, our previous observations suggest that TLR4-mediated signaling leading to NF-κB activation could be a novel pathway contributing to the development of cardiac hypertrophy in vivo (13, 21). Because MyD88 is an adaptor protein for TLR-mediated signaling leading to NF-κB activation, we evaluated the effect of blocking the MyD88-mediated signaling pathway on the cardiac hypertrophy in vivo. We constructed an adenovirus expressing dominant negative MyD88 (Ad5-MyD88) and transfected it into the myocardium immediately followed by banding the aorta for 3 wk. We have observed that transfection of this defective MyD88 into the myocardium significantly reduced aortic banding-induced cardiac hypertrophy, whereas transfection of Ad5-GFP did not. Transfection of Ad5-dnMyD88 into the myocardium significantly reduced aortic banding-increased NF-κB binding activity. In addition, we observed in our in vitro study that transfection of Ad5-dnMyD88 into rat cardiomyoblasts significantly inhibited NF-κB activation stimulated by LPS. Our observation is consistent with other reports showing that overexpression of dominant negative deletion mutants of MyD88 inhibited IL-1-induced activation of NF-κB (6, 15, 24, 30) and prevented TLR4-mediated NF-κB activation by LPS (10). Macrophages isolated from MyD88-deficient mice are completely defective in the production of an inflammatory response to the stimulation by bacterial components, suggesting that MyD88 plays a significant role in TLR-mediated signaling pathways (7, 10).

The role of cardiomyocyte apoptosis in the transition of the hypertrophic hearts to heart failure has been documented (9, 25, 29). However, the mechanisms by which cardiac myocyte apoptosis occurs during the development of cardiac hypertrophy are still unclear. Recent evidence suggests that MyD88 plays a role in mediating apoptosis (3, 9). Overexpression of MyD88 significantly induced a delayed cytotoxic effect in cardiomyocytes induced hypertrophy in vivo (8). We have previously identified as apoptosis-promoting proteins. Interestingly, we have observed that blockade of MyD88 by transfection of Ad5-dnMyD88 into the myocardium significantly attenuates aortic banding-induced cardiac hypertrophy in vivo, reduces cardiac myocyte apoptosis, and improves cardiac function following pressure overload. Our results suggest that MyD88 is an important participant in aortic banding-induced hypertrophy in vivo.

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DECREASED CARDIAC MYOCYTE DEATH BY BLOCKING MyD88


