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Bacillus Calmette-Guérin and TLR4 Agonist Prevent Cardiovascular Hypertrophy and Fibrosis by Regulating Immune Microenvironment

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Hypertension-induced cardiovascular hypertrophy and fibrosis are critical in the development of heart failure. The activity of TLRs has been found to be involved in the development of pressure overload-induced myocardial hypertrophy and cardiac fibrosis. We wondered whether vaccine bacillus Calmette-Guérin (BCG), which activated TLR4 to elicit immune responses, modulated the pressure overload-stimulated cardiovascular hypertrophy and cardiac fibrosis in the murine models of abdominal aortic constriction (AAC)-induced hypertension. Before or after AAC, animals received BCG, TLR4 agonist, IFN-γ, or TLR4 antagonist i.p. BCG and TLR4 agonist significantly prevented AAC-induced cardiovascular hypertrophy and reactive cardiac fibrosis with no changes in hemodynamics. Moreover, TLR4 antagonist reversed the BCG- and TLR4 agonist-induced actions of anti-cardiovascular hypertrophy and cardiac fibrosis. BCG decreased the expression of TLR2 or TLR4 on the heart tissue but TLR4 agonist increased the expression of TLR2 or TLR4 on the immune cells that infiltrate into the heart tissue. This led to an increased expression ratio of IFN-γ/TGF-β in the heart. The cardiac protective effects of BCG and TLR4 agonist are related to their regulation of ERK-Akt and p38-NF-κB signal pathways in the heart. In conclusion, the activity of TLR4 plays a critical role in the mediation of pressure overload-induced myocardial hypertrophy and fibrosis. The regulation of immune responses by BCG and TLR4 agonist has a great potential for the prevention and treatment of hypertension-induced myocardial hypertrophy and cardiac fibrosis. The Journal of Immunology, 2008, 180: 7349–7357.

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4 Abbreviations used in this paper: BCG, bacillus Calmette-Guérin; AAC, abdominal aortic constriction; LV, left ventricle; DC, dendritic cell; ASP, arterial systolic pressure; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; dp/dt, derivative of pressure.
Also, BCG has significantly beneficial non-targeted effects on general morbidity and mortality in low-income countries. The mechanisms behind the effects of BCG on general mortality and morbidity have been largely unexplored. Besides promoting a strong Th1 immune response via activation TLR4, BCG stimulates a Th2 immune response by targeting TLR2 and the C-type lectin DC-SIGN, which is responsible for \textit{Mycobacterium bovis} escaping immune surveillance (9). However, there is no study to show whether BCG has potential benefits in the prevention and treatment of cardiovascular hypertrophy and fibrosis.

In this study, we present evidence to support that the BCG vaccination or an adjuvant application of TLR4 agonist prevents cardiovascular hypertrophy and fibrosis following the sustained pressure overload. The beneficial effects of BCG or TLR4 agonist in hypertension are resulted from the activation of TLRs expressing in the DCs infiltrating into cardiovascular tissue in hypertensive animals. This indicates that BCG and TLR4 agonist are of great potential to be applied in the prevention and treatment of hypertension-induced myocardial hypertrophy and fibrosis.

Materials and Methods

\textbf{Animals}

All mice or rats were purchased from Vital River. The study protocol was approved by the Institutional Committee for the Ethics of Animal Care and Treatment. Male ICR mice were vaccinated i.p. with BCG (8.4 \times 10^5 CFU/kg; TianTan Biological Products) every 3 days for a week before abdominal aortic constriction (AAC). AAC was induced by aortic banding as previously reported (10). In brief, the aorta above the two renal arteries was dissected and a blunted needle was laid alongside the exposed aorta. A silk ligature was passed under the aorta and needle and tied securely. After the tie was completed, the needle was withdrawn. TLR4 antagonist msbB (140 g/kg; InvivoGen) or DC-SIGN antagonist Mannan (0.5 mg/mouse; Sigma-Aldrich) was administrated i.p. 1 hr before BCG. MsbB is an antagonist LPS from \textit{Escherichia coli} K12 msbB strain-TLR4 ligand, which lacks the myristoyl fatty acid moiety of the lipid A. The ability of msbB to inhibit TLR4 activity was previously confirmed (11). Mannan is a natural ligand for DC-SIGN, which can efficiently block the binding of HCV envelope glycoprotein E2 to the lectin domain of DC-SIGN molecule (12).

In the TLR4 agonist study, Wistar rats (male, 240 –280g) were randomized into the seven groups (18 rats/group) and underwent AAC or a sham operation after applying anesthesia with sodium pentobarbital (40 –50 mg/kg, i.p.) (3). Before AAC, some animals received i.p. IFN-\gamma (1.5 \times 10^7 U/kg/day) or Ec-LPS from \textit{E. coli} 0111:B4 strain (10 \mu g/kg/3 days) (InvivoGen) for 1 wk, whereas other rats were administered IFN-\gamma (1.5 \times 10^7 U/kg/day), Ec-LPS (10 \mu g/kg/3 days), or vehicle from day 1 to 28. For Ec-LPS function blocking, the rats were treated i.p. with 100 \mu g/kg/day of TLR4 antagonist 1 hr before Ec-LPS was injected from day 7 to 0. Our preliminary study showed that msbB (100 \mu g/kg/day) alone had no effect on cardiovascular hypertrophy (data not shown). Evidence shows that Ec-LPS only activates the TLR4 pathway (13).

\textbf{Echocardiography and hemodynamic assessment}

On day 28 of AAC, rats were anesthetized with i.p. injection of sodium pentobarbital (40–50 mg/kg). Interventricular septum and anterior end-systolic
**FIGURE 2.** BCG promoted the Th1/Th2 balance toward the Th1 immune responses. *a* and *d*, BCG inhibited expression of TGF-β1. Scale bar, 300 μm. *b* and *e*, BCG significantly increased expression of IFN-γ, which was inhibited by msbB. Scale bar, 300 μm. *c* and *g*, BCG significantly decreased expression of IL-10, which was inhibited by msbB. Scale bar, 300 μm. *f*, BCG increased the expression ratio of IFN-γ/TGF-β1. *h*, BCG decreased the expression of TLR2 and TLR4 in the pressure-overload heart. Scale bar, 100 μm. *i*, BCG decreased the number of the heart-infiltrating M2-macrophages (yellow color) in the left ventricular sections detected by two color confocal microscopy. The macrophages were costained with F4/80 and CD206 as indicated. Scale bar, 37.5 μm. Values were mean ± SEM (n = 6). ***, p < 0.01 vs sham group; **, p < 0.05, ***, p < 0.01 vs model group.

Histological analysis

Hearts and aortas were fixed in 4% paraformaldehyde, embedded in paraffin, and stained with H&E or Masson’s trichrome blue for analysis of hypertrophy and fibrosis. To determine cardiomyocyte size, the shortest transverse diameter was measured in the nucleated transverse section of the myocyte (n = 6 per group, 8 regions per heart, at least 50 cells per heart for size estimates) (14). To determine collagen deposition, sections stained with Masson’s trichrome were scanned and analyzed with a digital image analyser. Collagen fractions were calculated as the ratio of the collagen area and the total ventricular area in the corresponding section (n = 6 per group, 8 regions per heart). Immunohistochemical staining with 3,3'-diaminobenzidine was carried on paraffin-embedded slices. The sections were scanned at ×200 magnification. The images were then digitalized and the integrated OD of IFN-γ and TGF-β were calculated by Image-Pro plus5.1 software.

Confocal analysis

The heart specimens were prepared as described, and slides were incubated with specific two-mixtures of Abs: rabbit anti-TLR4, mouse anti-sarcomeric actin (cardiac myocytes), mouse anti-rats OX62L (dendritic cells, DCs), rabbit anti-mouse F4/80 (Santa Cruz Biotechnology), or rat anti-mouse CD206 (Serotec) Ab. Specific binding of primary Abs were detected using corresponding secondary FITC- or Rhodamine-conjugated goat anti-rabbit, FITC-conjugated goat anti-mouse/rabbit, or Rhodamine-conjugated goat anti-mouse/Fl (Santa Cruz Biotechnology). TLR4 expression, TLR4 colocalization with DCs, or cardiac myocytes and M2 macrophages expression (labeling F4/80 and CD206 simultaneously) (15) were examined using an E2000U confocal microscope and evaluated using Leica TCS SP2 software.

Quantitative RT-PCR and real-time quantitative PCR

Total RNA was isolated from the frozen left ventricle (LV) using the Trizol kit (Invitrogen) following the manufacturer’s instructions. Then the RNA was reverse-transcribed and amplified. PCR was performed using Mycycler thermal cycler and analyzed by agarose gel. Real-time quantitative PCR was performed using an ABI PRISM7000 Sequence detection system monitored by SYBR green. The specific primer sequences are as follows: mouse brain natriuretic peptide (BNP) 5'-AGGGAGACACGGGCATCATT-3' and 5'-GACAGACACCTTCAGGAGAT-3'; mice β-actin, 5'-GAATCGTGCTGTCATCAAAG-3' and 5'-TGTAATTTCATGGATGCCACAG-3'; atrial natriuretic peptide (ANP) 5'-ATCACCAAGGGCTTCTTCCT-3' and 5'-TGTGGACACCCGCACTGTAT-3'; TLR4, 5'-CGCTCTGGCATCATCTTCAT-3' and 5'-CTTTCAGGTCGAATGTCTTGC-3'; and rat β-actin, 5'-TACCACTGGGGAGGAGGAT-3' and 5'-AACACAGGAGGACACAGGCT-3'. All values obtained were normalized to the values obtained with the β-actin.

Western blot analysis

Cytoplasmic and nuclear fractions were prepared as described previously (14). Western blot was performed as described (8). Specific Ab binding was visualized by ECL (Amersham Biosciences).

Flow cytometry-intracellular cytokine staining

Single-cell suspensions of spleen cells were incubated overnight in RPMI 1640 medium. The cells were re-stimulated with PMA (20 ng/ml) and ionomycin (1 μg/ml) for 6 h and examined for intracellular IFN-γ and...
IL-4 accumulation. To prevent cytokine secretion, Monensin (1.7 μg/ml) was added to the culture for the final 4 h. Cells were then fixed (2% paraformaldehyde) and permeabilized (0.5% saponin). Intracellular IFN-γ was detected with PE-conjugated rat anti-mouse IFN-γ mAb and IL-4 was detected with FITC-conjugated rat anti-mouse IL-4 mAb. Data was collected and analyzed using Cellquest (BD Biosciences) or FlowJo (Tree Star).

**FIGURE 3.** TLR4 agonist prevented pressure overload-induced myocardial hypertrophy and cardiac fibrosis. 

- **a.** Representative example of the hearts. TLR4 agonist reduced the AAC-enlarged size of hearts. 
- **b and e.** The size of myocytes stained with H&E. Scale bar, 50 μm. 
- **c and g.** Histological analysis of thoracic aortas. TLR4 agonist blunted AAC-increased vascular wall diameter (Masson’s staining). Scale bar, 50 μm. 
- **d and h.** Cardiac fibrosis was assessed as the percentage of the heart section with collagen deposition, which was stained as bright blue. Scale bar, 100 μm. 
- **f and i.** The expression of ANP and collagen I mRNAs in the LVs (n = 5). 
- **j and k.** TLR4 agonist-decreased myocyte size was reversed by TLR4 antagonist msbB (H&E). Scale bar, 50 μm. 

Data was mean ± SEM (n = 6). **,** p < 0.01, **,** p < 0.001 vs sham group; *, p < 0.05, **,** p < 0.01, **,**,**,** p < 0.001 vs model group.

**Statistical analysis**

All values were presented as mean ± SEM. Statistical differences between groups were compared by ANOVA followed by a Tukey-Kramer’s or Dunnett’s multiple comparisons test, with p < 0.05 considered statistically significant. Unless specifically noted, analysis was performed with n = 6–8 per group in a given assay.
TLR4 agonist enhanced expression of TLR4 in the heart-infiltrating DCs in advance. The left ventricular sections were stained with Abs to TLR4 (green or red), OX62 (red, to visualize the DCs infiltrated into the hearts), and Actin (green, to visualize the cardiac myocyte) by two color confocal microscopy. TLR4 agonist increased the expression of TLR4 on the DCs. Scale bar, 37.5 μm.

FIGURE 5. TLR4 agonist ameliorated AAC-induced cardiovascular hypertrophy and cardiac fibrosis

Because TLR4 antagonist reversed the BCG-produced beneficial effects in cardiac hypertrophy or fibrosis, we were curious to see whether using TLR4 agonist alone was a good strategy to use in the prevention and treatment of the abnormal cardiac remodeling in hypertension using a rat model of AAC. BCG induced a significant cardiovascular hypertrophy, enlarged myocyte size, cardiac fibrosis, and interstitial fibrosis (Fig. 1, d–f). Coincidently, the BCG vaccination blocked the pressure overload-enhanced expression of BNP mRNA (Fig. 1g). Moreover, AAC stimulated a remarkable interstitial fibrosis (Fig. 1, e and h), whereas BCG significantly prevented the hypertension-induced cardiac fibrosis and decreased collagen accumulation (0.57% ± 0.24% in the BCG-treated AAC mice vs 2.14% ± 0.79% in the vehicle-treated AAC mice; p < 0.05). Pretreatment of AAC mice with TLR4 antagonist-msbB, but not with DC-SIGN inhibitor-Mannan, reversed the BCG-induced cardioprotective effects in the reduction of cardiac hypertrophy and fibrosis. BCG had no impact on the arterial systolic pressure (ASP) (Fig. 4f) and the heart rate (data not shown).

AAC induced a significant reduction in the ratio of IFN-γ/TGF-β in the hearts, whereas BCG treatment considerably increased the pressure overload—decreased ratio of IFN-γ/TGF-β (Fig. 2, a and b, d–f). Also, BCG significantly blocked AAC-induced expression of IL-10, TLR2, and TLR4 in the hearts (Fig. 2, c, g, and h). Accumulating evidence suggests that classically (M1)/alternatively (M2) activated macrophages mediate the Th1 or Th2 immune response respectively; the M2 macrophages have been found to be involved in the regulation of tissue remodeling through the mediation of Th2 immune response (15). We found that BCG prevented an AAC-induced increase in the number of heart-infiltrating M2-macrophages on day 28 after surgery (Fig. 2i). In addition, AAC induced a considerable reduction in the ratio of IFN-γ-producing cells/IL-4-producing cells (0.22% ± 0.08% vs 1.00% ± 0.21% in sham group) in the spleen, whereas BCG or BCG plus Mannan remarkably increased the ratio. Though BCG plus msbB also increased the ratio, the ratio was lower than that in BCG or BCG plus Mannan-treated mice (data not shown).

TLR4 agonist ameliorated AAC-induced cardiovascular hypertrophy and cardiac fibrosis

Results

BCG prevented cardiac hypertrophy and fibrosis by promoting the Th1 immune responses via activation of TLR4

AAC-resulted hypertension significantly enlarged the heart size of mice on day 28 of AAC (Fig. 1a). This was supported by a significant increase in the interventricular septum and anterior wall diameter at the end systole as well as an increase in myocyte diameter in the AAC mice (Fig. 1, b–d and f). By contrast, the BCG vaccination significantly protected the animals from the pressure overload-enhanced heart size (Fig. 1, a–c) as well as the enlarged myocardial size (Fig. 1, d and f). Coincidently, the BCG vaccination blocked the pressure overload-enhanced expression of BNP mRNA (Fig. 1g). Moreover, AAC stimulated a remarkable interstitial fibrosis (Fig. 1, e and h), whereas BCG significantly prevented the hypertension-induced cardiac fibrosis and decreased collagen accumulation (0.57% ± 0.24% in the BCG-treated AAC mice vs 2.14% ± 0.79% in the vehicle-treated AAC mice; p < 0.05). Pretreatment of AAC mice with TLR4 antagonist-msbB, but not with DC-SIGN inhibitor-Mannan, reversed the BCG-induced cardioprotective effects in the reduction of cardiac hypertrophy and fibrosis. BCG had no impact on the arterial systolic pressure (ASP) (Fig. 4f) and the heart rate (data not shown).

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TLR4 agonist ameliorated AAC-induced cardiovascular hypertrophy and cardiac fibrosis

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and significantly increased the fetal gene expression of ANP and collagen I (Fig. 3, a–i). By contrast, pretreatment or treatment of AAC rats with TLR4 agonist Ec-LPS significantly prevented and attenuated both the cardiovascular hypertrophy and cardiac fibrosis ($p < 0.05$). IFN-$\gamma$ showed an identical cardioprotective effect in the pressure overload-induced cardiovascular hypertrophy. IFN-$\gamma$ was used as an

FIGURE 6. TLR4 agonist promoted the Th1 response in LVs. TLR4 agonist increased the expression ratio of IFN-$\gamma$/TGF-$\beta$ in the hearts on days 7 (a), 14 (b), and 28 (c) after AAC. Data were representative of immunohistochemistry staining. Scale bar, 100 $\mu$m. Data were mean $\pm$ SEM ($n = 6$). ***, $p < 0.001$, ###, $p < 0.001$ vs sham group; **, $p < 0.01$, ##, $p < 0.01$ vs model group.

FIGURE 7. Regulatory effects of TLR4 agonist in the expression and activation of hypertrophy and fibrosis related signaling pathways. The left ventricular cytosolic lysates and nuclear protein were obtained from the animals on day 28 of AAC procedure. (a) Expression of Akt and GSK-3$\beta$. TLR4 agonist significantly inhibited the expression of Akt and increased the expression of GSK-3$\beta$. (b) Expression of p38 kinase. TLR4 agonist markedly reduced the expression and activity of p38. (c) Expression of ERK1/2 and NF-$\kappa$B. TLR4 agonist remarkably inhibited the expression and activity of ERK1/2 and NF-$\kappa$B. Data was folds of control $\pm$ SEM of four independent experiments. *, $p < 0.05$, **, $p < 0.01$ vs sham group; *, $p < 0.05$, **, $p < 0.01$ vs model group.
active control because the inhibitory effects of IFN-γ on myocardial hypertrophy have been recently reported (16).

The specific TLR4 antagonist msbB reversed Ec-LPS-induced reduction to the size of myocytes (Fig. 3, j and k). Also, msbB reversed Ec-LPS-induced anti-fibrotic effects; the ratio of collagen area in the msbB-pretreated rats had no difference from that in the vehicle-treated rats (p > 0.05) (Fig. 3, l and m). Suggesting that Ec-LPS prevented and attenuated the pressure overload-induced cardiac hypertrophy and fibrosis via the activation of the TLR4 signaling pathway.

TLR4 agonist prevented pressure overload cardiovascular hypertrophy and cardiac fibrosis were not due to the changes in hemodynamics in the AAC animals. AAC resulted in an increase in ASP by 50% on day 28 of AAC, as compared with the sham rats (p < 0.001) (Fig. 4). TLR4 agonist did not lower the ASP and heart rates in AAC rats as compared with the sham rats (Fig. 4, a and b). The derivative of pressure (dp/dt) max reflected the contractility of heart and was raised significantly in the AAC animals, but went unchanged or slightly declined in the agonist-treated animals (Fig. 4c). Diastolic function (dp/dt min) showed analogous disparities (Fig. 4, d and e).

**TLR4 agonist enhanced expression of TLR4 in the heart-infiltrating DCs and changed the balance of Th1/Th2 cytokines in the hearts**

To determine the precise location of TLR4 in the hearts, the left ventricular sections were analyzed with confocal microscopy. Expression of TLR4 was colocalized on the DCs infiltrating to the heart, but not on the cardiomyocytes (Fig. 5, a and b). Moreover, TLR4 agonist stimulated an increase in expression of TLR4 in the heart-infiltrating DCs. Interestingly, the expression of TLR4 mRNA was significantly increased in the Ec-LPS-treated rats on day 1 and in the vehicle-treated rats on day 3 (Fig. 5c). This suggested that the activation of TLR4 by Ec-LPS in advance prevented cardiac hypertrophy and fibrosis when compared with the vehicle-treated animals. We next investigated whether AAC changed the balance of Th1/Th2 cytokines in the heart on days 7, 14, and 28 after AAC or sham surgery. The expression of IFN-γ on LV cardiomyocytes was significantly increased in the Ec-LPS treated animals, while the expression of TGF-β was significantly decreased, indicating that Ec-LPS increased the expression ratio of IFN-γ/TGF-β on day 7 after AAC (Fig. 6a). The similar disparities were also observed on days 14 (Fig. 6b) and 28 (Fig. 6c) after AAC surgery. TLR4 antagonist msbB blocked these effects.

**Agonists of TLRs decreased expression of AKT, increased expression of GSK-3β, and blunted MAPK cascade activation in pressure-overload rats**

We examined whether activation of TLR4 had a crosstalk with the classical hypertrophy signals to modulate myocardial hypertrophy and cardiac fibrosis. As shown in Fig. 7a, TLR4 agonist significantly inhibited the expression and activity of AKT and enhanced the expression of TGF-β (p < 0.05). Expressions of p38, p-p38, ERK, and p-ERK in the rats treated with TLR4 agonist were markedly decreased in comparison to the vehicle-treated rats (Fig. 7, b and c). NF-κB is a major transcription factor to mediate of TLR4 activation. The activity of NF-κB was increased in the heart of vehicle-treated AAC rats, but was significantly decreased in the heart of TLR4 agonist-treated rats (Fig. 7c). These results indicated that cardiac protective effects of TLR4 agonist in the pressure-overload animals were associated with an inhibited expression and activity of Akt, an enhanced activity of GSK-3β, and a blunted MAPK activation.

**Discussion**

Active vaccination against self-molecules is emerging as an intriguing strategy for the treatment of chronic diseases (17). For instance, vaccination has been proposed in the treatment of cardiovascular diseases, including hypertension (18). In this study, BCG is used to vaccinate mice before AAC and to markedly prevent cardiac hypertrophy and fibrosis by the activation of TLR4 signaling that promotes the Th1 immune responses in the heart and spleen. This is the first time that BCG vaccine was used against abnormal cardiac remodeling following pressure overload. BCG-induced Th1 responses are inhibited by an antagonist of TLR4 but not by the antagonist of DC-SIGN-Mannan. This indicates that the cardioprotective effect of BCG in pressure-overload animals is specifically mediated by the activation of TLR4. Indeed, adjuvant application of TLR4 agonist alone attenuates pressure overload-induced myocardial hypertrophy and cardiac fibrosis. Our results seem apparently different from others in which the blockage of TLR4 activity or TLR4 signaling pathway provides cardiac protective effects, such as, attenuation of pressure overload-induced myocardial hypertrophy. The cardioprotective effects produced by the blockage of TLR4 are related to the inhibition of endogenous TLR4 activity-mediated inflammation which reduces myocardial hypertrophy; whereas BCG or TLR4 agonist-produced cardioprotective effects may be due to BCG or TLR4 agonist stimulating a nonspecific protective immune response that protects against detrimental effects of pressure overload on the cardiovascular system. Indeed, other work suggests that low levels of cytokines are protective for myocytes, but persistently high levels are detrimental (19). Whatever the mechanisms are, however, the fact that the blockage of TLR4 or activation of TLR4 by BCG or a low dose of TLR4 agonist induces a similar attenuation in myocardial hypertrophy strongly suggests that TLR4 activity is critical in the regulation of pressure overload-induced myocardial hypertrophy.

Pressure overload-induced cardiac fibrosis is a consequence of inflammatory response. Regulation of inflammation is a novel strategy to prevent myocardial fibrosis and resultant diastolic dysfunction in hypertensive hearts (20). Activation of TLRs directs the innate immune system’s responses, which in turn guide the subsequent adaptive immune reaction (21, 22) and particularly the balance of Th1 and Th2 responses. It has been found that fibrogenesis is strongly linked with the development of a Th2 response (23). IFN-γ is a critical Th1 cytokine, and TGF-β is a major Th2 cytokine. We found that BCG or a low dose of TLR4 agonist significantly increases the ratio of IFN-γ/TGF-β expression in the hearts following AAC, indicating that the mechanism underlying the cardioprotection of TLR4 activation was attributed to the shift of Th1/Th2 balance to Th1 response. By coincidence, Yu Q et al. (24) demonstrated that ventricular stiffness significantly increased the BALB/c whose immune function was Th2-predominate. It did not change in the C57 wild type whose immune function was Th1-predominate in the N(G)-nitro-1-arginine methyl ester inducing hypertensive mice. The total cardiac fibrillar collagen and percentage of fibrillar collagen cross-linking significantly increased in the BALB/c, and did not change in the C57 wild type. As Karl T. Weber (25) recently pointed out, “the road from inflammation to fibrosis is a stiff stretch of highway filled with aggressive immune cells and reconstruction sites, where delays in ventricular distensibility can be expected.” Modifying the behavior of these reckless cells may prove to be the best means of traffic control.

Because both cardiac myocytes and immune cells express TLR4, it is important to determine which cell type is responsible for TLR4 agonist- and BCG-induced cardioprotection following pressure overload. Earlier studies showed that TLR4 expresses in...
crosstalk with Akt and GSK-3. Activation of TLR4 attenuates myocardial hypertrophy via the PI3K/Akt signaling pathway, which is sufficient to confer sensitivity to mice lacking TLR4 in all other tissues (29). However, because inactivation of marrow-derived TLR4 function alone does not protect against LPS-triggered contractile dysfunction, TLR4 function in other tissues (including myocytes) may also contribute to this response. By labeling TLR4 and cardiac myocytes, or TLR4 and DCs simultaneously, we find that TLR4 was expressed in the heart-infiltrating DCs. Therefore, the anti-hypertrophy and anti-fibrosis actions of TLR4 agonists and BCG are most likely related to TLRs on DCs.

The PI3K/Akt signaling pathway plays a critical role in regulation of myocardial hypertrophy. Overexpression of PI3K or Akt leads to a significant increase in the heart weight/body weight ratio in mice (30, 31). Chronic over-expression of Akt is sufficient to produce pathological hypertrophy in transgenic mice (31). Akt is at a branch point of the signaling cascade and it signals downstream components to determine the nature of a given hypertrophic response. In this study, the expression of Akt and p-Akt is significantly decreased and the ratio of p-GSK-3β/GSK-3β is considerably increased in TLR4 agonist-treated hearts. This indicates that activation of TLR4 attenuates myocardial hypertrophy via the crosstalk with Akt and GSK-3β signaling pathways. However, it is unclear how activation of TLR signaling induces inhibition of PI3K/Akt and activation of GSK-3β. Ha et al. (3) found that cardiac hypertrophy was reduced in the TLR4-deficient mouse, which is related to the expression of lower level p-Akt in TLR4-deficient mice. The recent work of Hua et al. (32) indicates that protection against myocardial ischemia/reperfusion injury in the TLR4-deficient mice is mediated through a PI3K/Akt-dependent mechanism. The mechanisms by which PI3K/Akt are increased in the TLR4−/− myocardium may involve increased phosphorylation/inactivation of myocardial phosphatase and tensin homologue deleted in chromosome 10, as well as, increased phosphorylation/inactivation of myocardial GSK-3β (32). Fukao et al. (33) think that PI3K may be an endogenous negative feedback regulator that is crucial to the maintenance and integrity of the immune system. Thus, we speculated that in our study the activation of TLR4 on the immune cells induces a moderate immune response. This kind of protective response can prevent from pressure overload-increase the expression and activity of Akt and enhance the activity of GSK-3β (34). Additionally, we found that TLR4 agonist markedly decreases the expression of p38, p-p38, ERK1/2, p-ERK1/2, and the activity of NF-κB. Thus, TLR4 agonist-reversed cardiac remodeling is associated with the regulation of ERK-AP-1 and p38-NF-κB signaling pathways. However, it needs more research to determine the precise mechanism on how activation of TLR signaling induces inhibition of PI3K/Akt and the activation of GSK-3β. More research is also needed to determine how TLR agonists integrate more than one pathway into regulating myocardial hypertrophy and cardiac fibrosis.

In summary, the evidence that BCG vaccination and TLR4 agonist prevent pressure overload-induced cardiac remodeling by skewing the Th1 immune responses is of great clinical interest—given the high prevalence of hypertensive heart diseases and cardiac remodeling, which plays a critical role in several forms of heart failure.

Disclosures

The authors have no financial conflict of interest.

References


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