Signaling for myocardial depression in hemorrhagic shock:
roles of Toll-like receptor 4 and the p55 TNF-α receptor

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Running Title: Pro-inflammatory signaling and myocardial depression

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ABSTRACT

Hemorrhagic shock causes myocardial contractile depression. While this myocardial
disorder is associated with increased expression of tumor necrosis factor-alpha (TNF-α), the role
of TNF-α as a myocardial depressant factor in hemorrhagic shock remains to be determined.
Moreover, it is unclear which TNF-α receptor mediates the myocardial depressive effect of TNF-
α. Toll-like receptor 4 (TLR4) regulates cellular expression of pro-inflammatory mediators
following lipopolysaccharide stimulation and may be involved in tissue inflammatory response to
injury. The contribution of TLR4 signaling to tissue TNF-α response to hemorrhagic shock as
well as the role of TLR4 in myocardial depression during hemorrhagic shock are presently
unknown. We examined the relationship of TNF-α production with myocardial depression in a
mouse model of nonresuscitated hemorrhagic shock, assessed the influence of TLR4 mutation,
resulting in defective signaling, on TNF-α production and myocardial depression, and
determined the roles of TNF-α and TNF-α receptors in myocardial depression using a gene
knockout (KO) approach. Hemorrhagic shock resulted in an increase in plasma and myocardial
TNF-α (4.9 folds and 4.5 folds, respectively) at 30 min, while it induced myocardial contractile
depression at 4 h. TLR4 mutation abolished the TNF-α response and attenuated myocardial
depression (left ventricular developed pressure 43.0 ± 6.2 mmHg in TLR4 mutant vs. 30.0 ± 3.6
mmHg in wild type, P<0.05). TNF-α KO also attenuated myocardial depression in hemorrhagic
shock, and the p55 receptor KO, but not the p75 receptor KO, mimicked the effect of TNF-α KO.
The results suggest that TLR4 plays a novel role in signaling to the TNF-α response during
hemorrhagic shock and that TNF-α through the p55 receptor activates a pathway leading to myocardial depression. Thus, TLR4 and the p55 TNF-α receptor represent therapeutic targets for preservation of cardiac mechanical function during hemorrhagic shock.

Key words: TLR4, TNF-α, TNF-α receptors, hemorrhagic shock, mouse
INTRODUCTION

Tumor necrosis factor-alpha (TNF-α) is recognized as a cardiac depressant factor in septic shock and has been linked to heart failure associated with several different conditions (13, 37). Previous investigations have shown that transgenic overexpression of TNF-α results in congestive heart failure (8, 16). We have observed that TNF-α contributes to myocardial contractile dysfunction induced by either bacterial lipopolysaccharide (LPS) or myocardial ischemia/reperfusion (20-22). While TNF-α is involved in endotoxemic myocardial depression, there is a delay of at least 2 hrs between peak TNF-α production and myocardial depression (22). This observation suggests that TNF-α depresses myocardial contractility through the induction of downstream factors. Hemorrhagic shock also causes cardiac contractile dysfunction (15, 19), and this cardiodepressive effect is associated with the induction of TNF-α (2, 31, 35, 40). While TNF-α antibody has been shown to improve survival in hemorrhagic shock (5, 41), the mechanistic role of TNF-α in myocardial depression during hemorrhagic shock remains to be determined.

Innate immunity plays a primary role in acute host defense. Toll-like receptor 4 (TLR4) regulates cellular expression of pro-inflammatory mediators following LPS stimulation (27). LPS activates the TLR4 signaling cascade through the TLR4 TIR domain that uses a conserved signal transduction pathway requiring MyD88, IRAKs, IKK and NF-κB to induce a cytokine response (23). Indeed, TLR4 mediates cytokine production and myocardial depression in endotoxemia (6, 24). There are also data that suggest a broader role for TLR4 in tissue inflammatory response.
The contents of injured cells and the inducible heat shock protein 70 have been shown to provoke the inflammatory response through TLR4-mediated mechanisms in cultured mononuclear cells (4, 17, 38). Furthermore, TLR4 gene expression is up-regulated in the liver in a model of non-resuscitated, fixed-volume hemorrhagic shock (30). These findings suggest that TLR4 signaling may contribute to tissue inflammatory response to injury. However, the contribution of TLR4 signaling to tissue TNF-α response to hemorrhagic shock has not been determined, and the role of TLR4 in myocardial depression following hemorrhagic shock is presently unknown.

TNF-α signaling is mediated by two types of cell surface receptors (1, 11). The p55 TNF-α receptor is the dominant effector in TNF-α biology (26). While the p75 TNF-α receptor is known to play an important role in ligand passing to the p55 receptor (1), several studies have found that the p75 TNF-α receptor suppresses p55 TNF-α receptor function (25, 26). We have found that mice with deficient p55 TNF-α receptor have attenuated lung neutrophil accumulation following hemorrhagic shock (34). While pulmonary inflammatory response to hemorrhagic shock involves the p55 TNF-α receptor, it remains unclear which TNF-α receptor is involved in myocardial depression following hemorrhagic shock.

The purposes of this study were: 1) to examine whether TNF-α production temporarily correlates with myocardial depression in hemorrhagic shock, 2) to assess the effect of defective TLR4 signaling on the TNF-α response and myocardial depression during hemorrhagic shock, and 3) to test whether TNF-α deficiency or TNF-α receptor deficiency attenuates myocardial depression.
MATERIALS AND METHODS

Animals and Experimental Protocols

Male mice, body weight 20-25 grams, were used in this study. Wild type (BALB/cJ, B6/129 and C57 BL/6) and mutant (C.C3H-Tlr4<sup>lpr</sup>-d, TNF-α KO, p55 receptor KO and p75 receptor KO) mice were obtained from Jackson Laboratory (Bar Harbor, ME). The C.C3H-Tlr4<sup>lpr</sup>-d mouse strain is a constructed mutant that has a mutation on TLR4 TIR domain identical to that in C3H/HeJ (39). Since this mutant is constructed on BALB/cJ background, BALB/cJ mice were used as wild type controls. The background strain for TNF-α KO is B6/129, and the background strain for both p55 TNF-α receptor KO and p75 TNF-α receptor KO is C57 BL/6. The animals were quarantined and maintained on a standard pellet diet for a week before initiation of the experiments. All animal experiments were approved by the Animal Care and Research Committee, University of Colorado Health Sciences Center. Animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

The mouse hemorrhagic shock model has been described previously (33, 34). Mice were anesthetized with metofane. Hemorrhagic shock was induced by removing 30% of the calculated total blood volume (0.27 ml per 10 g of body weight), over 60 seconds, through a cardiac puncture. With this method, mean arterial pressure decreased to around 40 mmHg and remained in this low level for approximately 120 min (34). Overall mortality was less than 10%,
and there was no evidence of bleeding into the pericardial space and hemothorax, or signs of lung and cardiac contusion. The sham procedure involved cardiac puncture under metofane anesthesia, without removal of blood. Animals were sacrificed at 0.5, 1, 2 and 4 h following treatment. After anesthetization and heparinization (60 mg/kg of pentobarbital sodium and 2000 units/kg of heparin, ip), the chest was opened. For measurement of TNF-α, blood was collected from the right ventricle. The heart was removed and coronary vessels were flushed with 2.0 ml of cold normal saline through the aortic root. Plasma and myocardial samples were stored at –70 °C before TNF-α assay. Hearts were also isolated at 1, 2 and 4 h for the assessment of myocardial contractile function by Langendorff perfusion.

Isolated heart perfusion

Isolated heart perfusion was performed by a modified isovolumetric Langendorff technique as described elsewhere (28, 29). Hearts were excised into cold modified Krebs-Henseleit solution (in mM: 11 glucose, 119 NaCl, 1.2 CaCl₂, 4.7 KCl, 25 NaHCO₃, 1.18 KH₂PO₄ and 1.17 MgSO₄). The aorta was cannulated with a 20G needle, and the heart was perfused within 2 min after isolation. Hearts were perfused in an isovolumetric mode (70 mmHg) with the modified Krebs-Henseleit solution which was saturated with 92.5% O₂-7.5% CO₂ to achieve a PO₂ of 420-460 mmHg and a PCO₂ of 36-40 mmHg. The myocardial temperature was maintained by placing the heart in a jacketed tissue chamber that was kept at 37°C by circulating warm water. A thin latex balloon was inserted through the left atrium into the left ventricle, and
the balloon was filled with water to achieve a left ventricular end-diastolic pressure of 8 to 15 mmHg. Pacing wires were fixed to the right atrium, and the heart was paced at 5.0 Hz (300 beats/min) during perfusion. After 15 min of equilibration, left ventricular developed pressure (LVDP) and its first derivatives (+dP/dt and –dP/dt) were continuously recorded for 15 min using a computerized pressure amplifier/digitizer (Maclab 8, AD Instrument, Cupertino, CA).

**TNF-α assay**

Myocardial homogenate was prepared in 4 parts of homogenate buffer [0.1 M sodium phosphate (pH 7.5) containing 0.1% Triton X100, 2 mM EGTA, 1.0 mM benzamidine and 1.0 mM phenylmethylsulfonyl fluoride]. Centrifugation was carried out at 4°C, 1000 xg for 15 min with an Eppendorf centrifuge (model 5417R, Brinkmann Instruments, Inc., Westbury, NY). The supernatant was collected for the measurement of TNF-α. TNF-α levels in plasma and in myocardial homogenate was measured using a mouse ELISA kit as described previously (29, 34).

**Reverse transcriptase-polymerase chain reaction (RT-PCR)**

RT-PCR was performed to assess TNF-α gene transcription in the myocardium using previously described primers (3). Total RNA was extracted from myocardial homogenate by phenol/chloroform extraction (10). Two µg of isolated RNA was subjected to RT-PCR with AMV reverse transcriptase and random hexaoligonucleotides as previously described (32). RT-
PCR was carried out at 42 °C for 30 min followed by enzyme inactivation at 99 °C for 5 min.

PCR SuperMix containing Taq DNA polymerase (Gibco BRL, Gaithersburg, MD) was used to amplify cDNA obtained from the reverse transcription. Glyceraldehyde phosphodehydrogenase (GAPDH) mRNA expression was assessed for control. The amplified products were separated in a 1.5% agarose gel containing 0.5x Tris-Borate-EDTA buffer, pH 8.3, and 1% ethidium bromide.

**Immunoblotting**

Myocardial tissue was homogenized in cold homogenate buffer (50 mM PBS, pH 7.4, 1.0 mM benzamidine, 1.0 mM PMSF and 1% Triton X100). The homogenate was centrifuged at 800 xg for 10 min at 4 °C, and the supernatant was collected. Following determination of protein concentration, the supernatant was mixed with an equal volume of SDS sample buffer. Size fraction and transfer were performed as described previously (33). The membrane was incubated with a goat polyclonal antibody against TLR4 (from Santa Cruz Biotechnology, 1:200 dilution with PBS containing 0.05% Tween 20 and 5% dry milk) for 60 min and then with peroxidase-labeled rabbit anti-goat IgG (1:2000 dilution) for 45 min. After thorough wash with PBS, band was detected using ECL technique.
Statistical analysis

Data are presented as mean ± standard error (SE). ANOVA with a post hoc Bonferroni/Dunn test was performed to analyze differences between experimental groups. Statistical significance was accepted within 95% confidence limits.

RESULTS

Myocardial depression following hemorrhage

Cardiac contractile function at 1, 2 and 4 h was similar following sham treatment. Mean LVDP in sham groups was comparable to that in untreated mice (54.3 ± 3.7 mmHg). While myocardial contractility at 1 and 2 h following hemorrhage was not different from that of sham control, it was depressed at 4 h (Figures 1A and 1B). LVDP was 34.9 ± 2.8 mmHg at 4 h following hemorrhage (P<0.05 vs. sham 4 h, Figure 1A). Similarly, +dP/dt was attenuated at 4 h following hemorrhage (Figure 1B).

TNF-α production following hemorrhage

The temporal changes in TNF-α levels are shown in Figures 2A and 2B. Data obtained at 0.5 to 4 h after sham treatment was combined since TNF-α levels did not change over time after sham treatment.

Low levels of TNF-α were detected in plasma and the myocardium of sham-treated mice (Figures 2A and 2B). A moderate increase in TNF-α levels was found at 0.5 and 1 h following
hemorrhage (Figures 2A and 2B), while TNF-α was undetectable at 0.5 and 1 h following hemorrhage in either plasma or myocardium of TNF-α KO mice. TNF-α levels in plasma and the myocardium at 2 and 4 h following hemorrhage were not different from that of sham control. The increase in myocardial TNF-α level was accompanied by an elevation of myocardial TNF-α mRNA (Figure 2C).

*The effect of TLR4 mutation on TNF-α production*

In TLR4 mutant mice, low levels of TNF-α were also detected in plasma and the myocardium following sham treatment (Figures 3A and 3B). However, TNF-α levels did not change at either 30 min or 1 h following hemorrhage, and TNF-α levels at both time points were lower than those of wild type mice (Figures 3A and 3B).

*The effect of TLR4 mutation on myocardial depression*

Myocardial contractile function following sham treatment in TLR4 mutant mice was not different from that in sham-treated wild type mice (Figures 4A and 4B). However, myocardial depression caused by hemorrhagic shock was markedly attenuated in TLR4 mutant mice (Figures 4A and 4B). LVDP was 43.0 ± 6.2 mmHg in TLR4 mutant mice at 4 h following hemorrhage (P<0.05 vs. wild type hemorrhagic shock, Figure 4A), and it was slightly lower than that in sham-treated TLR4 mutant mice. Moreover, +dP/dt was improved in TLR4 mutant mice following hemorrhage (Figure 4B).
The effect of TNF-α KO on myocardial depression

Myocardial contractile function following sham treatment in TNF-α KO mice was not different from that in sham-treated wild type mice (Figures 5A and 5B). However, myocardial depression caused by hemorrhagic shock was attenuated in TNF-α KO mice (Figures 5A and 5B). LVDP was 47.0 ± 0.9 mmHg in TNF-α KO mice at 4 h following hemorrhage (P<0.05 vs. wild type hemorrhagic shock, Figure 5A). In addition, +dP/dt was improved in TNF-α KO mice following hemorrhage (Figure 5B).

The effect of TNF-α receptor KO on myocardial depression

Myocardial contractile function following sham treatment in the p55 TNF-α receptor (TNFR-I) KO mice and the p75 TNF-α receptor (TNFR-II) KO mice was not different from that in sham-treated wild type mice (Figures 6A and 6B). Similar to the effect of TNF-α KO, TNFR-I KO attenuated myocardial depression at 4 h following hemorrhage. LVDP was 44.4 ± 2.2 mmHg in TNFR-I KO mice following hemorrhage (P<0.05 vs. wild type hemorrhagic shock, Figure 6A). TNFR-I KO also improved +dP/dt following hemorrhage (Figure 6B). In contrast, TNFR-II KO had a minimal influence on myocardial contractile function following hemorrhage (Figures 6A and 6B). There was no change in myocardial TLR4 protein level in TNFR-I KO mice (Figure 6C).
DISCUSSION

This study demonstrates that TLR4 plays a novel role in the TNF-α response and myocardial depression following hemorrhagic shock and that TNF-α contributes to myocardial depression through the p55 receptor.

TNF-α has been demonstrated to be a cardiodepressant factor in a variety of animal models (20-22), and has been linked to heart failure in humans (13). In this study, we observed in a murine nonresuscitated hemorrhagic shock model that hemorrhage induces a rapid and transient increase in TNF-α levels in plasma and the myocardium. Both plasma and myocardial TNF-α levels peaked at 30 min and returned to baseline at 2 h following hemorrhage. The increase in myocardial TNF-α at 30 min is accompanied by an elevation of TNF-α mRNA, suggesting myocardium is a source of increased TNF-α in the heart. However, myocardial depression did not occur until 4 h. The time lag between the TNF-α response and myocardial depression suggests that TNF-α is an indirect cardiac depressant factor in this model of hemorrhagic shock. This delayed depression temporarily correlates with that observed in an endotoxemia model (22). Thus, myocardial depression induced by either hemorrhagic shock or LPS is a delayed disorder, and these two forms of stress may share a signaling mechanism.

Toll-like receptors play important roles in the innate immunoresponse. First identified as putative homologues of the Drosophila Toll protein, several members of the Toll-like receptor family have now been identified in mammals (7). Toll-like receptors transmit signals from the
ectodomain, consisting of multiple leucine-rich repeats, to the cytoplasm via the TIR domain. This process has been best described between LPS and TLR4 (27). In LPS-induced TLR4 signaling, the TIR domain uses a conserved signal transduction pathway requiring MyD88 (14). MyD88 interacts with TLR4 through its own carboxy-terminal TIR domain. Through its amino-terminal death domain, MyD88 recruits IRAK4 which phosphorylates IRAK1 to propagate the pro-inflammatory signal, leading to the phosphorylation of the IKK complex and MAPKs, including p38 and JNK (14). This phosphorylation event liberates bound NF-κB from the cytoplasm into the nucleus, resulting in the production of pro-inflammatory mediators. TLR4 seems to be involved in cellular response to injury (17). Interestingly, TLR4 gene expression is up-regulated in the liver in a similar model of non-resuscitated, fixed-volume hemorrhagic shock (30), and TLR4 signaling contributes to myocardial depression following burn injury (36). These findings suggest that TLR4 may play a role in the induction of the TNF-α response as well as in the promotion of myocardial depression following hemorrhagic shock. In the present study, we examined TNF-α response and myocardial depression in C.C3H-Tlr4<sup>lps-d</sup> mice that have a constructed mutation on the TIR domain, resulting in nonfunctional signaling defect (39). We found that TLR4 mutation abolished the TNF-α response and markedly attenuated myocardial depression following hemorrhagic shock. Thus, TLR4 plays a novel role in both TNF-α response and myocardial depression following hemorrhagic shock. However, TLR4-independent mechanisms appear to exist in myocardial depression since myocardial depression is attenuated, rather than abolished, in mice with defective TLR4.
It is unlikely that LPS is responsible for the TNF-α response during hemorrhagic shock since LPS is undetectable in the plasma and mesenteric lymph following hemorrhagic shock (12). TLR4 has been shown to be involved in cellular response to injury (17), and several studies have demonstrated that cellular endogenous proteins, such as heat shock protein 70, could signal through TLR4 to activate NF-κB (4, 38). These findings, therefore, indicate that TLR4 ligands may include endogenous agents. It remains to be determined whether TLR4 signaling cascade is activated during hemorrhagic shock and whether endogenous proteins mediate the inflammatory response to hemorrhagic shock.

In the present study, TNF-α KO markedly attenuates myocardial depression induced by hemorrhagic shock, thus confirming that TNF-α plays an important role in this disorder. However, TNF-α seems not to play a solo role in myocardial depression since TNF-α KO attenuates myocardial depression rather than abrogates it. TNF-α signaling is mediated by two types of cell surface receptors, the p55 TNF-α receptor and the p75 TNF-α receptor (11). We have found that mice lacking the p55 TNF-α receptor exhibit reduced lung neutrophil accumulation and attenuated lung injury following hemorrhagic shock (34). It is likely that TNF-α serves as a proximal mediator to initiate a pulmonary inflammatory cascade during hemorrhagic shock through the p55 receptor. In the present study, myocardial depression induced by hemorrhagic shock is attenuated by the p55 TNF-α receptor KO, suggesting that TNF-α signals through this receptor for the expression of depressant factors. Blockade of the p55 TNF-α receptor may protect cardiac function during stress that induces a TNF-α response.
It is unclear how TNF-α depresses myocardial contractility. TNF-α can directly depress myocardial contractility in vitro (9). Moreover, TNF-α has been shown to synergize with multiple cytokines to depress myocardial function (9, 18). However, it seems unlikely for TNF-α to be a direct depressant factor in myocardial depression in this model of hemorrhagic shock since plasma and myocardial TNF-α levels are normalized before myocardial depression occurs. Moreover, low concentrations of TNF-α alone are not sufficient to depress myocardial contractility (9). A spike in myocardial TNF-α level appears to serve as a signal, in the setting of hemorrhagic shock, to induce myocardial depressant factors. Determination of the role of TNF-α-induced factors in myocardial depression would suggest therapeutic approaches for myocardial protection during hemorrhagic shock.

Hemorrhage is an important factor in the etiology of end-organ failure associated with trauma (30). The results of the present study demonstrate in a murine model of hemorrhagic shock that hemorrhage alone triggers a pro-inflammatory signaling cascade and that an early pro-inflammatory response is involved in the induction of cardiac dysfunction. These findings suggest that suppression of the early pro-inflammatory response to hemorrhage is critical for preservation of cardiac function in trauma with severe hemorrhage. However, extrapolation of these observations to clinical situations should be made with caution since complex factors associated with trauma and resuscitation are absent in this a non-resuscitated hemorrhagic shock model.
ACKNOWLEDGMENTS

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REFERENCES


Figure 1. Myocardial depression during hemorrhagic shock. Wild type (B6/129) mice were subjected to hemorrhagic shock or sham treatment. Hearts were isolated at 1, 2 and 4 h after treatment, and left ventricular developed pressure (LVDP, A) and +dP/dt (B) were assessed by Langendorff perfusion. Myocardial depression occurred at 4 h after blood removal. Data are expressed as mean ± SE. n = 6 in each hemorrhagic shock group and n=4 in each sham group; * P<0.05 vs. sham control.
A

![Graph showing Plasma TNF (pg/ml) levels over time for Sham and HS groups.](image)

B

![Graph showing Myocardial TNF (pg/mg) levels over time for Sham and HS groups.](image)

C

![Image of gel showing mRNA expression of TNF-α and GAPDH.](image)

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Figure 2. Time course of TNF-α production. A and B. Plasma and myocardial samples were collected from wild type (B6/129) mice at 0.5, 1, 2 and 4 h following hemorrhage for the measurement of TNF-α levels by ELISA. Plasma and myocardial TNF-α increased at 0.5 h after hemorrhage. TNF-α ELISA data are expressed as mean ± SE. n = 6 in each time point; * P<0.05 vs. sham control. C. RT-PCR was performed on myocardial samples collected at 30 min following sham treatment (Sham) or hemorrhagic shock (HS) to examine TNF-α mRNA levels. The results confirmed an increase in myocardial TNF-α mRNA at 30 min.
Figure 3. The influence of TLR4 mutation on TNF production following hemorrhagic shock. Hemorrhagic shock was induced in wild type (BALB/cJ) and TLR4 defective mutant (C3H-Tlr4<sup>Lps-d</sup> strain, TLR4d) mice by removal of 30% of total blood volume. TNF-α in the plasma (A) and in the myocardium (B) was measured at 0.5 and 1 h following hemorrhage. TLR4 mutation abolished the TNF-α response. Data are mean ± SE. n = 6 in each group; * P<0.05 vs. BALB/cJ Sham and # P<0.05 vs. BALB/cJ HS.
Figure 4. The influence of TLR4 mutation on cardiac contractile dysfunction following hemorrhagic shock. Hemorrhagic shock was induced in wild type mice (BALB/cJ) and TLR4 defective mutant mice (C.C3H-Tlr4<sup>lpr-d</sup> strain, TLR4d) by removal of 30% of total blood volume. Hearts were isolated and perfused using the Langendorff technique 4 h after hemorrhage. Myocardial depression induced by hemorrhagic shock was attenuated in TLR4 mutants. Left ventricular developed pressure (LVDP) and +dP/dt are presented as mean ± SE. n = 5 in each group; * P<0.05 vs. BALB/cJ Sham and # P<0.05 vs. BALB/cJ HS.
Figure 5. The effect of TNF-α KO on myocardial depression. Hearts were isolated at 4 h after treatment, and left ventricular developed pressure (LVDP, A) and +dP/dt (B) were assessed by Langendorff perfusion. Myocardial depression induced by hemorrhagic shock (HS) was attenuated in TNF-α KO mice in comparison to that in wild type (WT, B6/129) mice although contractile function following sham-treatment in TNF-α KO mice was not different from that in wild type. Data are expressed as mean ± SE. n = 6 in each group; * P<0.05 vs. BALB/cJ Sham and # P<0.05 vs. BALB/cJ HS.
A

![LVDP Graph](image)

- **LVDP (mmHg)**
- **Sham**
- **HS**
- **WT**
- **TNFR-I KO**
- **TNFR-II KO**

B

![+dP/dt Graph](image)

- **+dP/dt (mmHg/s)**
- **Sham**
- **HS**
- **WT**
- **TNFR-I KO**
- **TNFR-II KO**

C

![Western Blot](image)

- **90 kD**
- **WT**
- **TNFR-I KO**
Figure 6. The effect of TNFR KO on myocardial depression and TLR4 level. A and B: Hearts were isolated at 4 h after hemorrhage or sham treatment, and left ventricular developed pressure (LVDP) and +dP/dt were assessed by Langendorff perfusion. Myocardial depression induced by hemorrhagic shock (HS) was attenuated in p55 TNF-α receptor (TNFR-I) KO mice in comparison to that in wild type (WT, C57BL/6) mice. In contrast, p75 TNF-α receptor (TNFR-II) KO had a minimal influence on myocardial depression. Data are expressed as mean ± SE. n = 6 in each group; * P<0.05 vs. Sham and # P<0.05 vs. WT HS. C: Myocardial samples collected from WT mice and TNFR-I KO mice were probed for TLR4 protein by immunoblotting. TNFR-I KO did not influence myocardial TLR4 level.