Downregulation of adenine nucleotide translocator 1 exacerbates tumor necrosis factor-α-mediated cardiac inflammatory responses

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Submitted 16 May 2014; accepted in final form 4 November 2014

Pan S, Wang N, Bisetto S, Yi B, Sheu SS. Downregulation of adenine nucleotide translocator isoform-1 (ANT1) protein levels were significantly decreased in the inflamed heart of C57BL/6 mice following cecal ligation and puncture. To understand the molecular mechanisms involved, we performed small-interfering RNA-mediated knockdown of ANT1 and studied tumor necrosis factor-α (TNFα)–induced inflammatory responses in myocardium-derived H9c2 cells and cardiomyocytes. ANT1 knockdown significantly increased swollen mitochondria and mitochondrial reactive oxygen species, concomitant with increased TNFα-induced NF-κB reporter gene activity and interleukin-6 and TNFα expression. A mitochondrial-targeted antioxidant mito-TEMPO attenuated TNFα–induced mitochondrial reactive oxygen species, NF-κB reporter gene activity, and cytokine expression in ANT1 knockdown cells. Interestingly, TNFα or lipopolysaccharide (LPS) treatment significantly decreased ANT1 protein levels, suggesting a feed-forward regulation of proinflammatory cytokine expression activated by ANT1 downregulation. These data suggest that ANT1 downregulation contributes to cardiac inflammation post-cecal ligation and puncture. Preventing ANT1 downregulation could provide a novel molecular target to temper cardiac inflammation.

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Materials and Methods

Animals. All animal experiments were conducted in accordance with experimental protocols approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University. Animals were maintained under pathogen-free condition (22 ± 0.5°C, 55% relative humidity, and 12-h:12-h light-dark cycle) at Thomas Jefferson University.

Antibodies. Vascular cell adhesion molecule-1 was from Santa Cruz Biotechnology (Dallas, TX), voltage-dependent anion channel (VDAC) was from Cell Signaling (Dancers, MA), and voltage-dependent anion channel-1 was from Dr. Douglas C. Wallace of the University of Missouri-Columbia (4).

Reagents. TNFα and IL-6 were from Roche Diagnostics (Indianapolis, MO), and anti-Fusion virus was from Dr. Christopher Baines of the University of Missouri-Columbia (45). NF-κB luciferase construct and TNFα small-interfering RNA (siRNA) were from Life Technologies (Grand Island, NY). Luciferase Reporter Assay System and β-galactosidase assay system were from Promega (Fitch, WI). Mito-TEMPO (MTP) was from Santa Cruz Biotechnology.

Mouse cecal ligation and puncture model. Male C57BL/6 mice of 8–12 wk old were used in the study. Mice were anesthetized with 1.5–2% isoflurane. The abdomens were shaved, and an ~1-cm midline incision was made. The cecum, isolated and ligated below the ileocecal valve without causing bowel obstruction, was punctured with a 22-gauge needle and was then gently squeezed to ensure that the holes were patent. The cecum was placed back in the abdomen, and the incision was closed with sutures. The mice were allowed to recover for at least 18 h, and then the mice were housed under the same conditions as the control group.
were then resuscitated with 1 ml of saline injected subcutaneously. Sham-operated controls were treated in an identical manner but without ligation and puncture. Survival was monitored every day for 72 h. Data were analyzed by log rank test using the statistical package R.

In vivo echocardiography. Transthoracic two-dimensional echocardiography was performed by using echocardiographic imaging system (Vevo 2100, VisualSonic, Toronto, Canada) with a 40-MHz probe. Mice were anesthetized through the inhalation of isoflurane (1–2%). M-mode interrogation was performed in the parasternal short-axis view at the level of the greatest left ventricular end-diastolic dimension. Left ventricular wall thickness and internal dimensions were measured and used for calculation. Fraction shortening and ejection fraction values were exported from the echo program.

Cell culture, siRNA oligonucleotide treatment, and adenovirus infection. Myocardial myoblast H9c2 cells were purchased from ATCC and cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum (Gibco, Grand Island, NY). Cells at passages 5–15 were used for experiments. Rat neonatal cardiomyocytes were isolated and cultured as previously described (7). To knock down ANT1, cells were treated with ANT1 siRNA. RNAiMAX (Life Technologies) was used according to the instructions of the manufacturer. Adenoviruses harboring mouse ANT1 were used to infect H9c2 cells as previously described (2).

Western blot analysis. Western blots analysis (immunoblots) were performed as previously described (35, 36). The images were acquired and analyzed by Licor Odyssey system. Equal loading of protein was ensured by measuring tubulin expression.

Quantitative real-time PCR. Qualitative real-time PCR was performed on iCycler iQ5 system using SYBR green (Bio-Rad). The primer sequences are as follows: TNFα, 5'-cccaacagcccctctgttc-3' and 5'-gtctctcctggaagaactg-3'; IL-6, 5'-tcagctctgctgtcct-3' and 5'-aaggcaagtgtggaagctct-3'; and 18S, 5'-tcagaaagcagttgaggag-3' and 5'-ggactcttaaggacatca-3'.

Reporter gene assay. Reporter gene and β-galactosidase activity assays were performed following the manufacturer’s instructions. Briefly, cells cotransfected with reporter gene construct, together with β-galactosidase construct, were treated with TNFα (10 ng/ml) for 18 h and harvested 48 h after transfection. Luciferase activity was measured following the manufacturer’s instructions. Data were normalized using β-galactosidase activity as the internal control.

Confocal and mitochondrial function assay. Cells were loaded with MitoTracker Green, MitoSox Red, or tetramethylrhodamine ethyl ester following the manufacturer’s instructions. Mitochondria images were acquired by Flouview (Olympus) using a ×60 oil objective and analyzed using National Institutes of Health ImageJ (v. 1.44). Oxygen consumption was measured using Clark electrode as previously described (45). Respiration control ratio was determined by stages 3 and 4 oxygen consumption.

Statistical analysis. Results represent at least three independent experiments if not mentioned specifically, and data are expressed as means ± SE. Where indicated, ANOVA was performed. One-way ANOVA was used for multiple group analysis, and paired Student’s t-test was used for the comparison between two groups. P < 0.05 was considered significant in all experiments.

RESULTS

ANT1 is downregulated in the inflamed heart. To understand the role for ANT1 in cardiac inflammation, we performed cecal ligation and puncture (CLP) in mice. Percent survival was significantly decreased after 24 h of CLP, likely because of CLP-induced organ failure (Fig. 1A). Therefore, we focused...
Fig. 2. Adenine nucleotide translocator isoform 1 (ANT1) is downregulated in the left ventricle of C57BL/6 mice after CLP. Protein extracts from the left ventricles of sham (s) or CLP mice (6-, 12-, and 24-h post-CLP) were analyzed for ANT1, cyclophilin D (CypD), and VCAM-1 protein levels by Western blot analysis. A: representative Western blot showing ANT1 protein levels after CLP. Protein extract from liver was loaded as the control, showing the antibody was specific for ANT1 (liver expresses ANT2). B: quantified results of A, expressed as the ratio to tubulin; n = 4. *P < 0.05 vs. sham. C: representative Western blot showing no change in CypD protein levels after CLP. D: quantified results of C, expressed as the ratio to tubulin; n = 4. P = 0.75 vs. sham. E: representative Western blot showing VCAM-1 protein levels after CLP. F: quantified results of E, expressed as the ratio to tubulin; n = 3. **P < 0.01 vs. sham.

Fig. 3. ANT1 knockdown results in a decrease in respiration control ratio and an increase in mitochondrial membrane potential in H9c2 cells. A: representative Western blot showing ANT1 knocking down by small-interfering RNA (siRNA). B: quantified results of A, expressed as the ratio to tubulin; n = 3. C: respiration control ratio in control and ANT1 knockdown cells; n = 3. D: mitochondrial membrane potential in control and ANT1 knockdown cells from 3 independent experiments. *P < 0.05 vs. control siRNA. TMRE, tetramethylrhodamine ethyl ester.
our studies on the initial 24-h post-CLP during which time inflammation was dominant. At 24 h, cardiac performance was significantly lower than that in sham-operated mice. The ejection fraction decreased from 66.1 ± 3.7 to 45.9 ± 18.5% (P < 0.05) and fraction shortening decreased from 35.6 ± 4.9% to 25.7 ± 16.9% (P < 0.05) as measured by echocardiography (Fig. 1, B and C). There was no significant change in heart rate at this 24 h time point (Fig. 1D). There was an ~50% decrease in cardiac output (Fig. 1E).

ANT1 protein levels were significantly decreased in the left ventricle of CLP mice at 24 h as assessed by Western blot (Fig. 2A). Protein extracts from liver were used as the control for antibody specificity (the ANT1 antibody detects only ANT1 in the heart, but not ANT2 in the liver) (Fig. 2A). In the heart, ANT1 decreased to 50% of sham at 24 h after CLP (Fig. 2B). In contrast, there was no significant difference in mitochondrial matrix protein CypD between sham and 24 h after CLP (Fig. 2, C and D). These data demonstrate that ANT1 is downregulated in the heart post CLP. Protein levels of vascular cell adhesion molecule-1, one of the indicators of inflammation, was increased ~2.4-fold at 24 h post CLP (Fig. 2, E and F).

ANT1 knockdown results in a reduction of mitochondrial respiration ratio and an increase in mitochondrial membrane potential in myocardium-derived H9c2 cells. To understand the effect of ANT1 downregulation on mitochondrial function, we performed siRNA studies to knock down ANT1 in H9c2 cells. ANT1 siRNA decreased ANT1 protein levels to 40% of control cells (Fig. 3, A and B) without affecting other mito-

![Figure 4](http://ajpheart.physiology.org/)
Antimicrobial proteins such as CypD (Fig. 3A, bottom) and voltage-dependent anion channel (data not shown). Mitochondrial respiration control ratio was decreased by ANT1 knockdown (from 7.4 ± 14.9% to 2.6 ± 18%, P < 0.05) (Fig. 3C). In addition, mitochondrial membrane potential was ~1.3-fold higher in ANT1 knockdown cells (Fig. 3D).

**ANT1 knockdown increases swollen and donut/blob-shaped mitochondria in H9c2 cells.** During sepsis, there is a marked and rapid increase in TNFα followed by other proinflammatory cytokines (38, 47). Therefore, to understand the meaning of ANT1 downregulation in inflammation, we studied TNFα-induced immune responses in ANT1 knockdown H9c2 cells.

We first studied the effect of ANT1 knockdown on TNFα-induced mitochondrial morphological changes by live cell confocal imaging. To compare mitochondrial morphology objectively among different groups of cells, individual mitochondria were measured in high magnification. Mitochondria are tubular under normal conditions but change to a donut shape under mild stress and to a blob shape under high stress (1). Control siRNA-transfected cells primarily displayed tubular and long mitochondria. The mitochondria form networks and spread throughout the cells (Fig. 4A). In contrast, ANT1 knockdown cells displayed an increased proportion of swollen and donut/blob-shaped mitochondria (Fig. 4C–E).

Because TNFα treatment causes the formation of donut/blob-shaped mitochondria (1), we next quantified whether ANT1 knockdown would favor TNFα-induced donut/blob-shaped mitochondria formation. In control siRNA-transfected cells, TNFα treatment significantly increased the percentage of donut/blob-shaped mitochondria (from 5.1 ± 0.2 to 14.4 ± 0.1, P < 0.05) (Fig. 4, B and E). Interestingly, ANT1 knockdown further increased donut/blob-shaped mitochondria (Fig. 4, C–E). In ANT1 knockdown cells, the percentage of donut/blob-shaped mitochondria was significantly increased after TNFα treatment (to 29.3 ± 0.05, P < 0.01 vs. matched control siRNA group), indicating that ANT1 knockdown sensitizes mitochondria to stress/TNFα-induced morphological changes (Fig. 4, C–E).

**ANT1 knockdown increases mitochondrial reactive oxygen species generation in H9c2 cells.** We next examined whether ANT1 knockdown increases mitochondrial reactive oxygen species (mtROS) because TNFα treatment induces mtROS generation (9). In control siRNA-transfected cells, TNFα treatment caused an ~2.0-fold increase in mtROS as indicated by the increased MitoSox Red fluorescent intensity (Fig. 5, A, B, and E). ANT1 knockdown alone (vehicle treatment) increased mtROS by ~2.0-fold (Fig. 5, C–E). Moreover, there was an ~5.0-fold increase in mtROS in ANT1 knockdown cells treated with TNFα (Fig. 5, D and E). Our data indicate that ANT1 downregulation promotes mtROS generation in H9c2 cells.

**ANT1 knockdown increases TNFα-induced NF-κB reporter gene activity and IL-6 and TNFα expression in H9c2 cells.** In both human patients and rodent models of sepsis, inflammation is associated with significant increases in ROS formation, NF-κB activation, and inflammatory cytokine production (3, 17, 42, 46, 50). Therefore, we next determined whether ANT1 modulates NF-κB reporter gene activity in response to TNFα.

In control siRNA-transfected cells, TNFα significantly increased NF-κB luciferase activity (from 19.7 ± 1.3 to 386.3 ± 1.7, P < 0.01) (Fig. 6A). More importantly, ANT1 knockdown further increased TNFα-induced NF-κB reporter gene activity (to 817.9 ± 1.3, P < 0.01 vs. TNFα-treated control group), suggesting that ANT1 knockdown enhances TNFα-induced NF-κB activation (Fig. 6A).

The role of ANT1 in NF-κB activation was further confirmed by infecting cells with either control adenovirus
Fig. 6. ANTI knockdown increases TNFα-induced NF-κB activation and IL-6 and TNFα expression in H9c2 cells. A: NF-κB luciferase reporter gene activity in ANTI knockdown cells. ANTI knockdown and control H9c2 cells were transfected with NF-κB luciferase reporter gene plasmid using transient β-galactosidase plasmid as the internal control. Cells were treated with 10 ng/ml TNFα for 18 h before harvest for assays. Results were from 3 independent experiments normalized by β-galactosidase activity as shown. *P < 0.01 vs. control siRNA treated with vehicle; **P < 0.01 vs. control siRNA treated with TNFα. AU, arbitrary units. B: NF-κB luciferase reporter gene activity in ANTI overexpressed cells. ANTI was transiently expressed in H9c2 cells using ANTI adenovirus (Ad-ANTI). Adenovirus expressing LacZ (Ad-LacZ) was used as the control. NF-κB luciferase activity was analyzed as in A. Data were from 3 independent experiments. *P < 0.01 vs. Ad-LacZ treated with vehicle; **P < 0.01 vs. Ad-LacZ treated with TNFα. Expression of IL-6 (C) and TNFα (D) in ANTI knockdown cells is shown. ANTI was knocked down by siRNA in H9c2 cells. Cells were treated with either vehicle or TNFα (10 ng/ml) for 8 h before being harvested at 72 h after siRNA treatment. mRNA was extracted from the cells, and IL-6 and TNFα expression were examined by quantitative real-time PCR analysis. Data from 3 independent experiments were normalized to control siRNA treated with vehicle. *P < 0.01 vs. control siRNA plus vehicle treatment; **P < 0.05 vs. control siRNA plus TNFα treatment.

(Ad-LacZ) or mouse ANTI adenovirus (Ad-ANTI) (Fig. 6B) (4). In control virus-transfected cells, TNFα significantly increased NF-κB luciferase activity (from 34.9 ± 3.1 to 748.9 ± 55.7, P < 0.01). More importantly, ANTI overexpression significantly reduced TNFα-induced NF-κB luciferase activity (to 50.1 ± 39.7, P < 0.01 vs. TNFα-treated control group) (Fig. 6B), supporting a positive role for ANTI downregulation in TNFα-induced NF-κB activation.

During inflammation, TNFα is the prime mediator of the inflammatory response and triggers the expression of other cytokines (47). Because TNFα and IL-6 are transcriptionally regulated by NF-κB, we next determined the effect of ANTI knockdown on the expression of IL-6 and TNFα in H9c2 cells (Fig. 6, C and D). There was an ~2.1-fold increase in endogenous IL-6 mRNA levels by ANTI knockdown alone (plus vehicle treatment). Moreover, the combination of ANTI knockdown and exogenous TNFα further caused an ~3.6-fold increase in IL-6 mRNA (Fig. 6C). Similarly, ANTI knockdown caused an ~1.97-fold and an ~10.1-fold increase in TNFα mRNA levels in vehicle- and recombinant TNFα-treated cells, respectively (Fig. 6D). Success in ANTI knockdown or overexpression was confirmed by Western blot analysis (Fig. 6, E and F).

MTP attenuates mitochondrial ROS generation, TNFα-induced NF-κB reporter gene activity, and IL-6 and TNFα expression in ANTI knockdown cells. Because ANTI knockdown increases mitochondrial-targeted antioxidant MTP would attenuate mtROS generation in control and ANTI knockdown cells in response to vehicle or TNFα (Fig. 7A). In control siRNA group treated with MTP, no significant change was detected in vehicle-treated cells. However, MTP significantly decreased TNFα-induced mtROS (from 1.5 ± 0.03 to 0.9 ± 0.02, P < 0.05). In ANTI knockdown group, MTP decreased mtROS in vehicle-treated cells (from 1.9 ± 0.03 to 0.9 ± 0.02, P < 0.05). More dramatic decrease of mtROS by MTP was found in TNFα-treated ANTI knockdown cells (from 2.5 ± 0.02 to 0.8 ± 0.05, P < 0.05) (Fig. 7A).
We next determined whether mtROS plays a role in TNFα-induced NF-κB activation in control and ANT1 knockdown H9c2 cells (Fig. 7B). In control siRNA group treated with MTP, TNFα-induced NF-κB reporter gene activity was significantly decreased (from 1508.2 ± 18.9 to 1158.9 ± 42.2, \( P < 0.05 \)). More dramatic changes of TNFα-induced NF-κB reporter gene activity were observed in ANT1 knockdown groups treated with MTP (from 3630.4 ± 184.9 to 2149.7 ± 80.7, \( P < 0.05 \)) (Fig. 7B).

We further determined whether MTP reduces TNFα-induced IL-6 and TNFα expression after ANT1 knockdown in H9c2 cells (Fig. 7, C and D). ANT1 knockdown increased TNFα-induced IL-6 and TNFα expression as we previously observed. MTP had little effect on the expression of IL-6 and TNFα in vehicle-treated control siRNA or ANT1 siRNA groups (MTP group vs. control group) (Fig. 7, C and D). However, in TNFα-treated cells, MTP decreased IL-6 expression in control siRNA groups (from 1508.2 ± 18.9 to 1158.9 ± 42.2, \( P < 0.05 \)) (Fig. 7C). More importantly, MTP decreased IL-6 expression in ANT1 siRNA groups treated with TNFα (from 3.5 ± 0.14 to 1.2 ± 0.01, \( P < 0.05 \)) (Fig. 7C). Similarly, MTP decreased TNFα expression in control...
siRNA groups treated with exogenous TNFα (from 3.7 ± 0.13 to 1.7 ± 0.01, P < 0.05) (Fig. 7D). Moreover, MTP dramatically decreased TNFα expression in ANT1 siRNA groups treated with exogenous TNFα (from 9.7 ± 0.88 to 1.7 ± 0.02, P < 0.05) (Fig. 7D).

The effect of MTP on IL-6 and TNFα expression was further confirmed using isolated cardiomyocytes (Fig. 7, E and F). ANT1 knockdown increased TNFα-induced IL-6 and TNFα expression (to 3.8 ± 0.4, P < 0.05, and 3.6 ± 0.3, P < 0.05, respectively) (Fig. E and F). MTP had little effect on the expression of IL-6 and TNFα in vehicle-treated control siRNA or ANT1 siRNA groups (MTP group vs. control group) (Fig. E and F). However, in TNFα-treated cells, MTP decreased IL-6 expression in control siRNA groups (from 1.4 ± 0.3 to 1.2 ± 0.3, P < 0.05) (Fig. 7E). More importantly, MTP significantly decreased IL-6 expression in ANT1 siRNA groups treated with TNFα (from 3.8 ± 0.4 to 1.1 ± 0.5, P < 0.05) (Fig. 7E). Similarly, MTP decreased TNFα expression in control siRNA groups treated with exogenous TNFα (from 1.6 ± 0.4 to 1.2 ± 0.3, P < 0.05) (Fig. 7F). Moreover, MTP dramatically decreased TNFα expression in ANT1 siRNA groups treated with exogenous TNFα (3.6 ± 0.3 to 1.1 ± 0.3, P < 0.05) (Fig. 7F). Together, our data support a role for mtROS in TNFα downregulation-induced inflammatory response, because mitochondria-targeted antioxidant MTP reduced NF-κB reporter gene activity, as well as IL-6 and TNFα expression stimulated by recombinant TNFα. These data are consistent with previous observations that TNFα causes the formation of donut/blob-shaped mitochondria and are supported by the finding that ANT1 negatively regulates ROS generation (1, 14, 23). Interestingly, TNFα and LPS treatments that mimic the environment in inflamed cells in turn decreased ANT1 protein levels, implicating a feed-forward regulation of inflammatory cytokine production mediated by ANT1 downregulation.

Our data support a model in which increased production of TNFα during CLP triggers ANT1 downregulation in the heart. In turn, ANT1 downregulation exacerbates TNFα-induced NF-κB activation and TNFα and IL-6 expression, leading to excessive inflammation in the heart (Fig. 8E).

During inflammation, immune factors such as cytokines and chemokines are secreted to coordinate defense and repair. However, excessive proinflammatory cytokines cause cellular and mitochondrial damage. In septic patients, one of the most severe and frequent complications is inflammation-induced cardiac dysfunction. Its presence is associated with significantly increased mortality rates (6, 31, 37, 51). Although the molecular mechanisms for sepsis-induced cardiac dysfunction are complicated and not completely understood, a growing

**DISCUSSION**

In the present study, we provide a novel target for mitochondrial regulation of cardiac inflammation. We found that ANT1 protein levels were significantly downregulated in the inflamed heart in our murine model of systemic inflammation induced by CLP. Characterization of mitochondria in ANT1 knockdown cells indicated a decrease in mitochondrial respiration control ratio, an increase in mitochondrial membrane potential and mtROS generation. Further mechanistic studies support a role for mtROS in ANT1 downregulation-induced inflammatory response, because mitochondria-targeted antioxidant MTP reduced NF-κB reporter gene activity, as well as IL-6 and TNFα expression stimulated by recombinant TNFα. These data are consistent with previous observations that TNFα causes the formation of donut/blob-shaped mitochondria and are supported by the finding that ANT1 negatively regulates ROS generation (1, 14, 23). Interestingly, TNFα and LPS treatments that mimic the environment in inflamed cells in turn decreased ANT1 protein levels, implicating a feed-forward regulation of inflammatory cytokine production mediated by ANT1 downregulation.

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**Fig. 8.** ANT1 is downregulated in TNFα or LPS treated H9c2 cells. H9c2 cells were treated with TNFα at 0, 10, and 50 ng/ml (A and B) or LPS at 250 ng/ml (C and D) for 24 h. ANT1 protein levels were examined by Western blot analysis. Data were from 3 independent experiments. *P < 0.01 vs. control; **P < 0.05 vs. control. E: model for the role of ANT1 downregulation in cardiac inflammation.
body of evidence indicates that increased inflammatory cytokine production contributes significantly to its pathogenesis (21, 30). Similarly, a sustained increase in TNFα or IL-6 has also been reported in several pathological processes, such as ischemic myocardial injury, cardiac hypertrophy, myocardial infarction, and chronic heart failure (5, 15, 19, 26, 49).

We demonstrate that ANTI downregulation enhances TNFα-induced NF-κB reporter gene activity. In septic patients, NF-κB is a critical indicator for survival because NF-κB activity is significantly higher in nonsurvivors and correlates strongly with the severity of the illness (3). The pathological role of NF-κB activation is also supported by the finding that inhibiting NF-κB prevents CLP-induced organ dysfunction and reduces cardiac hypertrophy and heart failure (8, 19). Interestingly, our data support a role for ANTI downregulation in mtROS-mediated NF-κB activation. Because sepsis-induced organ failure is associated with increased NF-κB activation and oxidative damage induced by mitochondrial dysfunction (10, 16), preventing ANTI downregulation could be beneficial, diminishing mtROS generation and NF-κB activation.

Studies to date indicate that mitochondria participate in the detection of microorganisms and cellular damage to activate innate immune responses (43, 44). Here, we provide further evidence for ANTI modulation of cardiac inflammation and assert the importance of cross talk between mitochondrial proteins and cytosolic pathways in the regulation of inflammation. Our data suggest that ANTI downregulation, NF-κB activation, and subsequent inflammatory cytokine production form a vicious cycle that exacerbates inflammation.

Although how much of an effect increasing myocardial ANTI would have in CLP mice is still not clear at this point. ANTI downregulation could be a novel molecular target in sepsis-induced cardiac inflammation, especially since mitochondrial mass and protein synthesis do not change during sepsis (16). Further studies on the regulatory mechanisms of ANTI will shed light on its role in preventing excessive inflammation.

ACKNOWLEDGMENTS

The ANTI-encoding adenovirus was a generous gift form Dr. Christopher Baines of the University of Missouri-Columbia. The ANTI antibody was a generous gift from Dr. Douglas C. Wallace of the University of Pennsylvania.

GRANTS

This work is supported by National Heart, Lung, and Blood Institute Grants HL110371 and HL093671 (to S. S. Sheu).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.P. conception and design of research; S.P., N.W., S.B., and B.Y. performed experiments; S.P. and N.W. analyzed data; S.P. and S.-S.S. interpreted results of experiments; S.P. and N.W. prepared figures; S.P. drafted manuscript; S.F., N.W., and S.-S.S. edited and revised manuscript; S.P., N.W., B.Y., and S.-S.S. approved final version of manuscript.

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ANTI1 DOWNREGULATION EXACERBATES INFLAMMATION


