Right ventricular TNF resistance during endotoxemia: the differential effects on ventricular function

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Markel TA, Crisostomo PR, Wang M, Herrmann JL, Abarbanell AM, Meldrum DR. Right ventricular TNF resistance during endotoxemia: the differential effects on ventricular function. Am J Physiol Regul Integr Comp Physiol 293: R1893–R1897, 2007. First published August 22, 2007; doi:10.1152/ajpregu.00359.2007.—Right and left ventricular myocytes originate from different cellular progenitors; however, it is unknown whether these cells differ in their response to endotoxemia. We hypothesized that 1) the percentage of endotoxemic functional depression within the right ventricle (RV) would be smaller than that of the left ventricle; and 2) that better RV function would correlate with lower levels of right ventricular TNF production. Adult Sprague-Dawley rats were divided into right and left control and endotoxin groups. Controls received vehicle, while endotoxin groups received LPS at 20 mg/kg ip. Hearts were excised either 2 or 6 h after injection. Hearts excised at 2 h were assayed for TNF, IL-6, TNF receptor 1 (TNFR1), TNFR2, and via ELISA, while hearts excised at 6 h were assayed via the Langendorff model. The percentage of cardiac functional depression, exhibited as developed pressure, contractility, and rate of relaxation (expressed as a percentage of control) was significantly smaller in right ventricles compared with left ventricles following endotoxin exposure. Tissue levels of TNF were significantly elevated in both right and left ventricles 2 h after endotoxin exposure, and right ventricular endotoxin groups expressed higher levels of TNF compared with their left ventricular counterparts. No significant differences in IL-6, TNFR1, or TNFR2 levels were noted between endotoxin-exposed ventricles. This is the first study to demonstrate that right and left ventricular function differs after endotoxin exposure.

Sepsis; cardiac; IL-6; cardiovascular collapse

Sepsis, viewed as an extensive version of the acute inflammatory response, has led to increased rates of morbidity and mortality despite advances in clinical and critical care medicine. In the United States, there are over a half-million cases of sepsis annually, with a death rate of 35–65% (20). Polymicrobial sepsis and organ dysfunction are associated with immunosuppression, a predominance of proinflammatory cytokines, including TNF-α and IL-6, as well as a profound loss of lymphocytes via apoptosis (3, 4, 7–9, 19, 25). In addition, various toxins are released from bacteria during sepsis, which serve to further promote cytokine release, vasodilation, oxidative damage, and subsequent cardiovascular collapse (13, 17, 23, 27).

Previous studies from our group have demonstrated that endotoxin induces left ventricular contractile dysfunction, and that certain chemical reagents, including p38 MAPK inhibitors and heat shock protein, can reduce this dysfunction (22, 29). Furthermore, endotoxin preconditioning has been shown to protect cardiac tissue, and it appears that estrogen and the MAPK pathways play a role in this mechanism (2, 15, 24, 30).

Right heart failure, however, is distinctly different from left heart failure and remains an understudied but important component of cardiovascular collapse (26). Recent studies have suggested that the heart forms from two distinctly different progenitor cell populations, known as “heart fields.” The primary heart field contributes to the left ventricle (LV) and atria, while the secondary heart field contributes to the right ventricle (RV) and outflow tract (1, 6, 11, 14). Cells from these heart fields are distinguished by specific cellular markers and transcription factors. Primary heart field cells are marked by the T-box transcription factor Tbx5 and the bHLH transcription factor Hand1. Secondary heart field cells are distinguished by the presence of Hand2, the LIM-homeodomain transcription factor Isl1, and Fgf10 (11). Functional genes that encode for proteins that aid in myocardial contraction, such as desmin and the ventricular specific myosin light chain MLC2V show expression in the right ventricle and outflow tract rather than the left ventricle (10). Furthermore, other genes such as the homeobox gene Nkx2-5 are expressed in both heart fields but rely on distinctly different regulatory elements for expression (11). Therefore, endotoxin might logically provoke different responses in these two “distinctly different” types of ventricular cardiomyocytes.

Endotoxin-induced right ventricular dysfunction has previously not been well characterized. Studies on pigs have demonstrated that RV function actually increases during acute endotoxin exposure, and only after energetic costs and myocardial oxygen are consumed does RV function begin to decrease (16). Previous studies have not directly compared the left and right ventricular responses to endotoxin, and therefore, a study of such nature would be useful to determine whether one ventricle’s function is affected more so than the other. We hypothesized that 1) the percentage of endotoxemic functional depression within the right ventricle would be smaller than that of the left ventricle; and 2) better RV function would correlate with lower levels of right ventricular TNF production.

MATERIALS AND METHODS

Animals. Age-matched (275–350 g) normal male Sprague-Dawley rats (Harlan, Indianapolis, IN) were fed a standard diet and acclimated
in a quiet quarantine room for 1 wk before the experiments. The animal protocol was reviewed and approved by the Indiana Animal Care and Use Committee of the Indiana University. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 85–23, revised 1985).

**Isolated heart preparation (Langendorff).** Rats were anesthetized (pentobarbital sodium, 60 mg/kg ip) and heparinized (500 U ip), and hearts were rapidly excised via median sternotomy and placed in 4°C Krebs-Henseleit solution [119 mM NaCl, 20.8 mM NaHCO₃, 11 mM dextrose, 12 mM CaCl₂(2H₂O), 47 mM KCl, 11.7 mM MgSO₄(7H₂O), and 11.8 mM KH₂PO₄]. The aorta was cannulated, and the heart was perfused under constant pressure (mean 75 mmHg) with oxygenated (95% O₂-5% CO₂) Krebs-Henseleit solution (37°C). A left or right atrial resection was performed before insertion of a water-filled latex balloon through the atrium into the ventricle. The balloon was initially adjusted to a desired mean end-diastolic pressure (EDP) of 5 mmHg, and hearts were allowed to equilibrate for 10 min. Pacing wires were fixed to the atrium, and hearts were paced at 6 Hz, 3V, 2 ms (350 bpm) throughout the study to ensure a standard heart rate between groups. Coronary flow was measured by collecting pulmonary artery effluent. Data were continuously recorded using a PowerLab 8 preamplifier/digitizer (AD Instruments, Milford, MA) and an Apple G4 PowerPC computer (Apple Computer, Cupertino, CA). The developed pressure and maximal positive and negative values of the first derivative of pressure (+dp/dt and −dp/dt) were recorded at EDPs of 5, 10, 20, 30, and 40 mmHg.

**Experimental isolated heart groups.** Rat hearts were divided into the following groups (n = 9 per group): 1) left ventricular control, 2) right ventricular control, 3) left ventricular endotoxin, and 4) right ventricular endotoxin. Rats selected as controls received an intraperitoneal vehicle injection of 1 ml PBS, while those selected for endotoxin injection received 20 mg/kg LPS ip (Salmonella typhimurium LPS; Sigma, St. Louis, MO) dissolved in PBS. Two hours after injection, several animals (n = 4 LV and RV controls, n = 4 LV and RV endotoxins) were anesthetized as above, and hearts were rapidly excised and flushed in retrograde fashion through the aorta with cold Krebs-Henseleit solution. The lateral walls of the left and right ventricles were immediately excised and snap frozen in liquid nitrogen for tissue cytokine analysis. Six hours after injection, the remaining animals (n = 5 per group) underwent isolated heart preparation via Langendorff, as previously described.

**ELISA.** The presence of TNF, IL-6, TNF receptor 1 (TNFR1), and TNFR2 within control and endotoxin exposed right and left ventricular tissue was determined by ELISA using commercially available ELISA sets (R&D Systems, Minneapolis, MN and BD Biosciences, San Diego, CA). Heart tissue was homogenized with cold lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₂VO₄, 1 μg/ml leupeptin, and 1 mM PMSF. ELISA was performed according to the manufacturer’s instructions. All samples and standards were measured in duplicate.

**Presentation of data and statistical analysis.** All reported values are expressed as means ± SE, and P < 0.05 was considered statistically significant.

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**Fig. 1.** Endotoxin (ETX) significantly depressed right and left ventricular function as demonstrated by decreased right and left ventricular developed pressure (A and D) +dp/dt (B and E), and −dp/dt (C and F). However, in the face of endotoxin exposure, right ventricular function was maintained at a higher percentage of baseline function compared with left ventricular function (G–I). *P < 0.05.
cally significant. Data were compared using Student’s t-test or two-way ANOVA with post hoc Bonferroni.

RESULTS

The percent of right ventricular functional depression during endotoxemia is smaller than that of the left ventricle. Twenty milligrams per kilogram of endotoxin significantly depressed both right and left ventricular function as measured 6 h after injection via Langendorff. Endotoxin-induced dysfunction was noted by a significant decrease in right and left ventricular developed pressure (Fig. 1, A and D), contractility (Fig. 1, B and E), and rate of relaxation (Fig. 1, C and F) at all measured EDPs. Maximum depression occurred at an EDP of 5 mmHg for both right (41.94 ± 2.59 to 21.14 ± 1.60 mmHg, \( P < 0.001 \)) and left ventricles (106.86 ± 4.42 to 33.53 ± 3.45 mmHg, \( P < 0.01 \)).

Interestingly, the percent of right ventricular functional depression was smaller than that of the left ventricle during endotoxemia. Right ventricular developed pressure, \( +\ dp/\ dt \) and \( -\ dp/\ dt \), expressed as a percentage of control, were significantly higher after endotoxin exposure compared with the LV (Fig. 1, G–I). The greatest difference in systolic function between right and left endotoxin-exposed hearts was noted in the percent preservation of developed pressure at EDP = 5 mmHg (right 50.42 ± 3.82% vs. left 31.378 ± 3.23%).

Coronary flow was also significantly reduced in all endotoxin groups compared with controls (Fig. 2A), and hearts selected for right or left ventricular analysis were injured equally with LPS (Fig. 2B).

Endotoxin elevates tissue levels of TNF. TNF levels from endotoxin-exposed right and left ventricles were significantly higher than their respective controls 2 h after endotoxin exposure. Interestingly, endotoxin-exposed right ventricles expressed higher levels of TNF (picogram per milligram ventricular tissue) compared with left ventricles (RV: 218.0 ± 27.12 vs. LV: 117.9 ± 6.77, \( P < 0.05 \)) (Fig. 3A). In contrast, IL-6 levels were not elevated in endotoxin-exposed right or left ventricles compared with controls. There was also no significant difference in IL-6 levels between right and left endotoxin-exposed ventricles (Fig. 3B).

Endotoxin elevates ventricular TNFR2 levels, but not TNFR1 levels. TNFR2, but not TNFR1, was significantly elevated in endotoxin hearts compared with controls. No significant difference, however, was observed in TNFR1 or TNFR2 levels between right and left ventricles after endotoxin exposure (Fig. 4, A and B).

DISCUSSION

Because of the prevalence of cardiovascular collapse during sepsis and endotoxemia, endotoxin-induced cardiac dysfunction is of particular interest. Further, the National Heart, Lung, and Blood Institute noted a shortage of scientific studies of the right heart and a paucity of information concerning its dysfunction. Herein, we demonstrated that endotoxin has a less profound effect on right ventricular function compared with left ventricular function, as evidenced by a smaller percentage of right ventricular functional depression during endotoxemia. Furthermore, TNF levels were actually observed to be elevated in right ventricular tissue, which may suggest a degree of TNF resistance in the right ventricle of the heart.

We noted that both right and left ventricular function were decreased with endotoxin exposure, as evidenced by impaired developed pressure, \( +\ dp/\ dt \), and \( -\ dp/\ dt \). However, the amount of functional depression (expressed as a percentage of control) was noted to be less in right ventricles. This was clearly seen in developed pressure, but was not as obvious in \( +\ dp/\ dt \) (contractility) or \( -\ dp/\ dt \) (rate of relaxation). Percentage of right ventricular contractility and rate of relaxation tended to be higher at all EDPs, but only certain points maintained statistical significance. The lack of statistical significance at other points may be due to limitations in the sensitivity of the Langendorff model.

TNF was found to be elevated in hearts exposed to endotoxin. Specifically, TNF levels were elevated in ventricles 2 h after endotoxin injection. A 2-h postinjection time point was chosen to capture peak levels of TNF expression. Moreover, RVs exposed to endotoxin maintained higher levels of TNF than LVs. On the other hand, IL-6 was not significantly elevated in ventricles 2 h after endotoxin injection. IL-6, which functions as an acute phase reactant, was expected to be elevated in endotoxemic animals. As TNF often serves to
further elicit the acute phase response, it is possible that this model of endotoxemia requires a longer period of TNF exposure to promote differences in IL-6 expression.

Elevated proinflammatory cytokines, including TNF, have previously been shown to be detrimental to cardiac function (21). Under physiological conditions, TNF effectively activates both of its receptors, TNFR1 and TNFR2 (5). However, during states of inflammation, one TNF receptor may be preferentially activated. TNFR1 activation results in an elevated proinflammatory response and decreased organ function after injury (21, 28), while TNFR2 activation has been shown to initiate protective signaling cascades (12, 18).

In this study, we actually observed a smaller percentage of functional depression in right ventricles after endotoxin exposure despite elevated TNF levels in right ventricular tissue and equivalent levels of TNF receptors between right and left ventricles. Therefore, it is possible that right ventricular myocytes may be more resistant to TNFR1 signaling, which may allow the right ventricle to function optimally in the presence of endotoxemia.

Right ventricular TNF signaling resistance may be due to a number of factors. First, as previously mentioned, right and left ventricular myocytes originate from different progenitor cells. Previous studies have shown different transcriptional regulatory sequences within right and left ventricles (10). Right ventricular cells may therefore retain different signaling mechanisms and intracellular pathways that allow them to perform better in the presence of endotoxins and proinflammatory cytokines. Another possible reason for TNF resistance is the size difference between right and left ventricles. As the LV has six times the mass of the RV, it has a larger cellular makeup that may contain more receptors capable of responding to endotoxins and proinflammatory cytokines. Further studies are therefore needed to elucidate the mechanisms of right ventricular endotoxin and cytokine tolerance. Understanding the mechanisms that the right ventricle uses to maintain better function in the face of endotoxemia may allow for the design and implementation of various therapies to attenuate endotoxemia-induced cardiovascular collapse.

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