Beta 2 Microglobulin Knockout Mice Treated Anti-AsialoGM1 Exhibit Improved Hemodynamics and Cardiac Contractile Function during Acute Intra-abdominal Sepsis

Weike Tao¹,³ and Edward R. Sherwood¹,²

Department of Anesthesiology, The University of Texas Medical Branch¹ and the Shriners Hospital for Children², Galveston, Texas

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³Current address: Department of Anesthesiology and Pain Management, The University of Texas Southwestern Medical Center, Dallas, Texas²

Address correspondence to: Edward R. Sherwood, M.D., Ph.D., Department of Anesthesiology, The University of Texas Medical Branch, Galveston, Texas 77555-0591, Tel. 409-772-1221, Fax 409-772-1224, Email. ERSherwo@UTMB.edu

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Abstract

We previously showed that β2 microglobulin knockout mice treated with anti-asialoGM1 (β2M/αAsGM1 mice) exhibit less hypothermia, reduced production of pro-inflammatory cytokines, less metabolic acidosis and improved survival following cecal ligation and puncture (CLP) compared to wild type mice. The present study was designed to assess hemodynamics and left ventricular contractility at 18 hours after CLP. Arterial pressure was measured by carotid artery cannulation and left ventricular pressure-volume loops were obtained by insertion of a 1.4F conductance catheter into the left ventricle. Heart rate, stroke volume and cardiac output were not significantly different between wild type and β2M/αAsGM1 mice following CLP. However, β2M/αAsGM1 mice exhibited improved mean arterial pressure and systemic vascular resistance compared to wild type mice. Myocardial function was also better preserved in β2M/αAsGM1 mice as indicated by improved left ventricular pressure development over time, time varying maximum elastance, end systolic pressure volume relationship and preload recruitable stroke work. Overall, this study shows that cardiovascular collapse characterized by hypotension, myocardial depression and low systemic vascular resistance occurs after CLP in wild type mice. However, β2M/αAsGM1 mice exhibit improved hemodynamics and cardiac contractile function following CLP that may account, in part, for our previously observed survival benefit.

Keywords: cecal ligation and puncture, blood pressure, vascular resistance, cardiac output, pressure-volume relationships
Introduction

We recently reported that β2 microglobulin knockout mice treated with anti-asialoGM1 (β2M/αAsGM1 mice) are resistant to acute intraabdominal sepsis caused by cecal ligation and puncture (CLP)(32). β2-microglobulin knockout mice express dysfunctional class I major histocompatibility (MHC-I) and CD1 complexes since β2 microglobulin comprises the β chain of these antigen-presenting molecules (3;5). Furthermore, CD8\(^+\) T and natural killer T (NKT) cells interact with antigens presented in association with MHC-I and CD1, respectively, and require the presence of these molecules for normal growth and differentiation (24;29;30). Therefore, β2-microglobulin knockout mice are also deficient in CD8\(^+\) T and NKT cells. Treatment of β2 microglobulin knockout mice with anti-asialoGM1 will cause further depletion of natural killer (NK) cells. Therefore, β2M/αAsGM1 mice possess multiple immunologic defects. However, our previous studies (32) show that the decreased susceptibility of β2M/αAsGM1 mice to septic peritonitis is due, in part, to depletion of CD8\(^+\) T and natural killer cells. β2M/αAsGM1 mice exhibit improved survival, less hypothermia, reduced production of pro-inflammatory cytokines and decreased metabolic acidosis compared to wild type mice subjected to CLP. Because of the acuity of our model, wild type mice did not show evidence of acute organ failure. Therefore, the underlying cause of mortality in wild type mice during acute intra-abdominal sepsis was not apparent in our initial study.

Hemodynamic alterations and cardiac contractile dysfunction are key manifestations of sepsis. Classically, septic patients exhibit hypotension, tachy-
cardia, decreased systemic vascular resistance, myocardial depression and impaired tissue perfusion (16;27;28). In fact, many of these alterations are used to define sepsis and septic shock (4). However, it is difficult to measure cardiovascular alterations in mice due to size limitations, making it difficult to fully interpret the clinical relevance of experimental sepsis models that use mice. Recently, some investigators have used echocardiography, microspheres or flow probes to measure hemodynamic function in septic mice (13;38). These techniques have provided useful information on hemodynamic function in mice during sepsis. However, they do not allow for accurate assessment of cardiac function in the face of low systemic vascular resistance. Left ventricular function may be better assessed using parameters that are not load dependent. We recently reported our studies on hemodynamic function in septic mice (42). A 1.4F left ventricular conductance catheter was employed in combination with carotid artery cannulation to measure hemodynamics and left ventricular function in mice after CLP. Advantages of using a left ventricular conductance catheter are the ability to resuscitate mice to a common left ventricular end-diastolic volume prior to performing hemodynamic measurements and to assess load insensitive indicators of left ventricular contractility by measuring the left ventricular pressure-volume relationship during transient inferior vena cava occlusion.

In order to gain insight into the physiologic mechanisms associated with resistance of $\beta_2 M/\alpha$AsGM1 mice to lethal CLP, we compared hemodynamics and cardiac contractile function in wild type and $\beta_2 M/\alpha$AsGM1 mice after CLP. We demonstrate that wild type mice exhibit cardiovascular collapse characterized
by hypotension, decreased systemic vascular resistance and myocardial depression by 18 hours after CLP. These cardiovascular alterations were less severe in β2M/αAsGM1 mice indicating that these mice are resistant to the hemodynamic alterations caused by acute intra-abdominal sepsis.
Materials and Methods

Mice. Female, 6-8 week old C57BL/6J and β2 microglobulin knockout (β2M−/−, strain B6.129P-B2m1Unc) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Natural killer (NK) cells were depleted by treatment of mice with anti-asialoGM1 (50 μg, intraperitoneal (IP), Cedarlane Laboratories, Hornby, Ontario, Canada) 24 hours prior to CLP. Treatment of mice with antibody against the surface glycolipid asialoGM1 causes targeted depletion of NK cells (32) (41). All studies were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch and conformed to NIH Guidelines for the Care and Use of Experimental Animals.

Cecal ligation and puncture. Mice were anesthetized with 2% isoflurane in oxygen via facemask. A 1-2 cm midline incision was made through the abdominal wall; the cecum was identified and ligated with a 3-0 silk tie 1 cm from the tip. Care was taken not to cause bowel obstruction. A single puncture of the cecal wall was performed with a 20-gauge needle. The cecum was lightly squeezed to express a small amount of stool from the puncture site in order to assure complete perforation. The cecum was returned to the abdominal cavity and the incision was closed with a continuous suture. Mice received 2 ml of lactated Ringer’s solution subcutaneously in the dorsal area for fluid resuscitation. Sham mice underwent anesthesia, laparotomy and wound closure but were not exposed to the cecal ligation and puncture procedure. Sham procedures were performed on both wild type and β2M/αAsGM1 mice. Comparison of hemodynamic and cardiac function data between these groups did not show significant differences. The
data presented in this report is from wild type mice that underwent sham procedures. Hemodynamic measurements were performed at 18 hours after sham (n = 10) or CLP procedures in wild type (n = 6) and β2M/αAsGM1 (n = 10) mice. Mice did not exhibit mortality within 18 hours after CLP in these studies.

**Left ventricular pressure-volume and systemic blood pressure measurements:** Mice were anesthetized with 2% inhaled isoflurane in oxygen as described above. Septic mice typically required a lower concentration of isoflurane compared to sham, thus adjustment was made based on the overall appearance and responsiveness of mice during the surgical preparation, but all data were collected at a standardized inhaled isoflurane concentration of 1.5%. A temperature probe (YSI 400, Level 1, Rockland, MA) was inserted into the rectum for initial temperature measurement by a digital thermometer (TM 2000D, RSP, Irvine, CA) as soon as mice were anesthetized. Mice were then placed on the procedure bench with heating pads (T/Pump, Gaymar, Orchard Park, NY) and lamps to maintain the body temperature between 36 and 37.5 °C.

The surgical procedure was performed under an operating microscope (OPMI-1, Karl Zeiss, Thornwood, NY). A small incision was made in the anterior neck to expose the trachea and the left carotid artery. The trachea was incised and intubated with an endotracheal tube modified from a 20-gauge intravenous catheter. The animal was then placed on positive pressure ventilation using a rodent ventilator (Model 683, Harvard, South Natick, MA) with a tidal volume of 400-450 μL and rate of 80-95 breaths per minute. These values were adjusted based on the animal’s carbon dioxide production guided by expired gas cap-
nometry (Capnomac Ultima, Tewksbury, MA). The left carotid artery was exposed and cannulated with stretched PE-10 tubing and transduced to an analog pressure module (Hewlett-Packard, Model M1175A, Waltham, MA). The analog signal of the mean arterial pressure (MAP) was interfaced with the data acquisition system described below.

A clamshell incision was made over the anterior chest wall 2 mm above the level of the xyphoid process. The chest cavity was entered with small mosquito clamps that were then applied across the sternum. The sternum was transected with an electrocautery system (Jorvet E1, Jorgensen Laboratories, Loveland, CO) and the mediastinum exposed. A 4-0 silk tie was passed around the inferior vena cava (IVC) just above the diaphragm. The pericardium was excised at the left ventricular apex. The apex was then punctured with a 27½-gauge needle occluded with bone wax. A 1.4 Fr catheter with pressure and conductance sensors (SPR 839, Millar Instruments, Houston, TX) was passed through the puncture along the longitudinal axis of the left ventricle. Left ventricular volume was estimated using the impedance/conductance technique. The conductance system is comprised of four electrodes. The proximal electrode generates a constant current that, along with the distal ground electrode, creates an electrical field within the cylindrical left ventricle. The voltage potential between the inner two electrodes is measured as the ventricle contracts and relaxes allowing for estimation of left ventricular volume. The catheter’s angle and position were adjusted to obtain optimized flow-volume curves. Specifically, measurements were made at the position from which the maximum volume measurement was obtained.
The 1.4F catheter also contains a single pressure sensor that allows continuous monitoring of left ventricular pressures.

The conductance catheter was interfaced with a pressure-volume analog signal amplifier (Aria 1, Millar Instruments). Real-time conductance and mean arterial pressure data were collected with an analog-to-digital converter (Power Lab 4SP, ADInstruments, Castle Hill, Australia). All variables were displayed and recorded (Chart ver. 4.12, ADInstruments). Initial pressure volume curves were assessed. In order to standardize the preload, 50-100 μL of normal saline was slowly given via the arterial line to reach a left ventricular end-diastolic volume between 15 and 18 relative volume units (RVU), which corresponded to 40-50 μL. The arbitrary RVU number was converted to true volume using calibration wells with known diameters of 2-6 mm (Millar Instruments) containing heparinized blood from mice of each group heated to 37 °C. Volume measurements were performed using blood from three mice in each experimental group. No significant differences in RVU measurements were observed between groups at any of the well diameters. These determinations were made to assure that differences in blood resistivity did not exist between groups. The target range of the left ventricular end-diastolic volume of 40-50 μL was chosen as the end-point of resuscitation and used as the standardized condition for hemodynamic measurements because it was found to be typical of healthy animals undergoing the same procedure with minimal or no blood loss in our pilot experiments.

Left ventricular pressure and volume were measured first at the steady state without IVC occlusion. Heart rate, stroke volume, cardiac output, as well as
pressure development during isovolumic contraction (dp/dt\textsubscript{max}) and relaxation (dp/dt\textsubscript{min}), were also obtained. After the steady-state measurements were performed, IVC occlusion was performed by gently lifting the tie around the inferior vena cava while briefly suspending mechanical ventilation. Left ventricular end-systolic pressure volume relationship (ESPVR) was obtained for each individual animal at the varying preload status produced by IVC occlusion. Time-varying maximum elastance (Emax) was obtained from the series of pressure-volume relationship regression curves at various preloads. Likewise, preload-recruitable stroke work (PRSW) data were generated with the varying left ventricular end-diastolic volumes. The slopes of the curves for all animals in the group were numerically averaged. To eliminate parallel conductance and resultant contribution of volume from the myocardium, a calibration with 10 µL of 15% hypertonic saline was conducted as described by Yang et al (43).

**Statistics.** Data are expressed as mean ± standard error of the mean. Calculation of dp/dt\textsubscript{max}, dp/dt\textsubscript{min}, Emax, ESPVR and PRSW were performed using PVAN software (Millar Instruments, Houston, Texas). Comparisons among groups were performed by one-way analysis of variance followed by a post hoc Tukey’s test. A value of p < 0.05 was considered statistically significant.
Results

The hemodynamic response of $\beta$2M/$\alpha$AsGM1 mice following cecal ligation and puncture. Mean arterial blood pressure was measured in wild type and $\beta$2M/$\alpha$AsGM1 mice immediately after cannulation of the carotid artery and prior to placement of the left ventricular conductance catheter (Figure 1). Mean arterial blood pressure was significantly lower in wild type mice at 18 hours after CLP compared to sham mice. Mean arterial pressure in $\beta$2M/$\alpha$AsGM1 mice did not differ significantly at 18 hours after CLP compared to sham mice and was significantly higher when compared to septic wild type mice (Figure 1).

Additional hemodynamic measurements were made after placement of the left ventricular conductance catheter and resuscitation of mice to left ventricular end diastolic volumes of 40-50 $\mu$l (Figure 2). Due to third space fluid redistribution and small blood losses associated with surgical manipulation, mean arterial blood pressure was slightly lower in mice after placement of the left ventricular conductance catheter compared to the pre-catheterization state. However, mean arterial pressure returned to values that were not significantly different from precatheterization levels after fluid resuscitation (Figures 1 and 2). Septic wild type mice had significantly lower mean arterial pressure after fluid resuscitation compared to sham mice. Mean arterial blood pressure was also significantly lower in $\beta$2M/$\alpha$AsGM1 mice compared to the sham group but was significantly higher than in septic wild type mice (Figure 2). Heart rate measurements were not significantly different between sham mice and septic wild type or $\beta$2M/$\alpha$AsGM1 mice (Figure 2). Stroke volume was significantly increased in wild type and
β2M/αAsGM1 mice at 18 hours after CLP compared to the sham group (Figure 2). No significant difference in stroke volume was observed when comparing septic wild type and β2M/αAsGM1 mice. Cardiac output was also significantly increased in septic wild type and β2M/αAsGM1 mice compared to sham mice (Figure 2). Comparison of wild type and β2M/αAsGM1 mice at 18 hours after CLP did not reveal significant differences in cardiac output. Systemic vascular resistance was significantly decreased in septic wild type mice compared to the sham group (Figure 2). β2M/αAsGM1 mice also exhibited a significant decrease in systemic vascular resistance following CLP compared to sham mice. However, systemic vascular resistance was significantly higher in septic β2M/αAsGM1 mice compared to the wild type group (Figure 2).

**Cardiac contractile function in β2M/αAsGM1 mice following cecal ligation and puncture.** Cardiac contractile function was determined in sham and septic mice both during the steady state and following transient occlusion of the inferior vena cava. Representative left ventricular pressure volume curves for sham, septic wild type and septic β2M/αAsGM1 mice are shown in Figure 3. Assessment of pressure development over time during contraction and relaxation (dp/dt<sub>max</sub> and dp/dt<sub>min</sub>) of the left ventricle was measured during the steady state (Figure 4). Wild type mice showed significant decreases in dp/dt<sub>max</sub> and dp/dt<sub>min</sub> at 18 hours after CLP compared to sham mice (Figure 4A). Comparison of sham and β2M/αAsGM1 mice after CLP did not reveal significant differences in dp/dt<sub>max</sub> but dp/dt<sub>min</sub> was significantly different in β2M/αAsGM1 mice compared to sham controls. Both dp/dt<sub>max</sub> and dp/dt<sub>min</sub> were significantly improved in β2M/αAsGM1 mice
compared to wild type mice at 18 hours after CLP. Because dp/dt\text{max} is a relatively preload sensitive marker of contractility, dp/dt\text{max} was corrected for left ventricular end diastolic volume (LVEDV) (Figure 4B). Both wild type and β2M/αAsGM1 mice exhibited significant decreases in dp/dt\text{max}/LVEDV at 18 hours after CLP compared to sham mice. However, dp/dt\text{max}/LVEDV determinations were significantly higher in β2M/αAsGM1 mice compared to wild type mice (Figure 4B).

Load-insensitive indicators of left ventricular contractility such as the end systolic pressure volume relationship (ESPVR), time varying maximum elastance (E\text{max}) and preload-recruitable stroke work (PRSW) were determined by measuring the left ventricular pressure-volume relationship during transient inferior vena cava occlusion (Figure 5). ESPVR was significantly lower in septic wild type and β2M/αAsGM1 mice compared to the sham group (Figure 5A). In addition, β2M/αAsGM1 mice exhibited a significantly higher ESPVR at 18 hours after CLP compared to wild type mice. E\text{max} was also significantly lower in wild type and β2M/αAsGM1 mice at 18 hours after CLP compared to sham mice (Figure 5B). Furthermore, β2M/αAsGM1 mice exhibited significantly higher E\text{max} at 18 hours after CLP compared to wild type mice. PRSW was significantly lower in septic wild type mice compared to sham controls (Figure 5C). PRSW in β2M/αAsGM1 mice was not significantly different compared to sham mice or septic wild type mice.
Discussion

The present study shows that $\beta 2M/\alpha$AsGM1 mice have less hemodynamic dysfunction compared to wild type mice following CLP. Specifically, $\beta 2M/\alpha$AsGM1 mice exhibit less myocardial depression and higher systemic vascular resistance. Both of these factors can explain the improved systemic arterial blood pressure observed in $\beta 2M/\alpha$AsGM1 mice. We previously reported that $\beta 2M/\alpha$AsGM1 mice exhibit less metabolic acidosis and improved survival compared to wild type mice after CLP (32). We also showed that $\beta 2M/\alpha$AsGM1 mice produce smaller amounts of pro-inflammatory cytokines such as interleukin (IL)-1$\beta$, macrophage inflammatory protein (MIP)-2 and IL-6 when compared to wild type mice. Pro-inflammatory cytokines are known to directly contribute to sepsis-induced myocardial depression and other alterations in hemodynamic function observed in septic patients as well as in experimental models of sepsis (9;17;23;25). Most prior studies have focused on macrophages and monocytes as the primary cell types driving the pro-inflammatory response during sepsis (8;10;21). In our prior report, we showed that depletion of NK and CD8$^+$T cells significantly contributes to the resistance of $\beta 2M/\alpha$AsGM1 mice to CLP-induced mortality. Because $\beta 2M/\alpha$AsGM1 mice exhibit a markedly decreased pro-inflammatory response following CLP, it is postulated that NK and CD8$^+$T cells contribute to the CLP-induced inflammation in mice. The importance of NK cells in potentiating inflammation caused by $E. coli$ or endotoxin challenge has been previously reported (2;11;39). NK cells are a major source of interferon (IFN)-$\gamma$ after administration of endotoxin or gram negative bacteria (39;40) (11). IFN-$\gamma$ acti-
vates macrophage and monocyte functions such as secretion of pro-inflammatory cytokines, antigen presentation and phagocytosis (33). Production of IFN-γ by NK cells contributes to the development of shock following endotoxin or *E. coli* challenge due to its ability to amplify the production of pro-inflammatory mediators such as tumor necrosis factor (TNF)α and IL-1 (2;11).

The contribution of CD8⁺T cells to the pathogenesis of septic shock is less well recognized. Chang and colleagues (7) showed that CD8⁺T cells contribute to *P. berghei*-induced shock in mice. Recent reports show that CD8⁺T cells are activated during the early phases of bacterial infection to produce IFN-γ by mechanisms that are similar to those causing induction of IFN-γ by NK cells (14;20). CD8⁺T cell proliferation and cytotoxicity can also be induced early after bacterial infection (36;37). Production of IFN-γ and direct cytotoxic effects of both CD8⁺T and NK cells may contribute to the pathophysiology of septic shock. The impact of these cell populations on the development of hemodynamic and cardiac contractile alterations described in this report remain to be fully ascertained. However, it should be pointed out that depletion of NK and CD8⁺T cells may only partly account for the resistance of β2M/αAsGM1 mice to acute intra-abdominal sepsis. β2M knockout mice have multiple immunologic defects including the absence of functional MHC-I and CD1 molecules in addition to natural killer T and CD8⁺T cell deficiencies(24;29;30). The contribution of each factor to the pathogenesis of lethal intra-abdominal sepsis remains to be fully established.

Results of this study show that hypotension, myocardial depression and decreased systemic vascular resistance occur in wild type mice following CLP.
These alterations are accompanied by increased cardiac output that is due primarily to augmented stroke volume in adequately resuscitated mice. Our previous studies show that mortality in wild type mice occurs most commonly at 18 to 30 hours after CLP and correlates with severe metabolic acidosis and hypothermia (32). Evidence of acute organ injury was not observed in our prior study. Taken together, these data suggest that mortality in mice exposed to rapidly lethal CLP is due to cardiovascular collapse, hypoperfusion and shock. The short interval between CLP and mortality in this model does not allow for the manifestation of the multi-organ dysfunction syndrome. Therefore, this model mimics the clinical scenario of rapidly progressive sepsis caused by acute bowel perforation with fecal spillage. The cardiovascular response to sepsis in humans is characterized by high cardiac output, low systemic vascular resistance and hypotension (1;26). Adequate volume resuscitation is required to manifest this hyperdynamic circulatory state. Paradoxically, numerous investigators have also described sepsis-associated myocardial depression characterized by decreased stroke work and left ventricular ejection fraction (9). The underlying cause of these derangements is thought to be hyperactivation of the pro-inflammatory response with systemic or local production of pro-inflammatory cytokines, platelet activating factor, eicosanoids and nitric oxide. Several studies have demonstrated that TNFα, IL-1 and nitric oxide have direct depressant effects on the myocardium and are likely to play a role in sepsis-induced myocardial dysfunction (6;18;19;22). The better preserved hemodynamic and cardiac contractile function shown in the present study coupled with attenuated pro-inflammatory cytokine re-
lease in our previous study in β2M/αAsGM1 mice further support the notion of cytokine-associated myocardial depression. Overall, this study shows that the cardiovascular derangements observed in wild type mice following CLP closely mimic those observed in septic humans, suggesting that this is a relevant model of septic shock (4). This is in agreement with the findings of Hollenberg et al (13) who showed, using echocardiography, that mice develop hyperdynamic sepsis following CLP, if adequately fluid resuscitated. Our study extends their findings by showing decreased systemic vascular resistance and myocardial depression in wild type mice following CLP.

Measurement of left ventricular pressures and volumes provides the ability to determine left ventricular pressure-volume relationships at different preloads achieved by IVC occlusion (31) (12) (34). This approach also allows for determination of variables such as the end-systolic pressure-volume relationship, maximum left ventricular elastance, and preload-recruitable stroke work that are believed to be load-insensitive indicators of myocardial contractility (12) (34) (35). The intact animal model also allows measurement of cardiac function in the presence of circulating cytokines, autonomic nervous system contributions, and humeral factors during sepsis. In contrast, there are some disadvantages to the use of the left ventricular conductance catheter through the open-chest approach. The surgical procedure predisposes mice to hypothermia and evaporative fluid losses. These factors were minimized in the present study by the use of warming techniques and administration of fluids. In addition, the initial IVC occlusion maneuver allows the investigator to perform measurements of left ventricu-
lar function before compensatory mechanisms are activated (15). However, repeated measurements may be difficult to interpret as cardiovascular reflexes become activated and may alter myocardial function. All data reported in this paper were obtained at the time of initial IVC occlusion in order to exclude the impact of autonomic reflexes on our results.

In conclusion, mortality of wild type mice following acutely lethal CLP is due primarily to cardiovascular collapse and tissue hypoperfusion. β2M/αAsGM1 mice exhibit less alteration of hemodynamics and left ventricular contractility after CLP compared to wild type mice. These findings, in conjunction with our previous report of decreased metabolic acidosis and reduced pro-inflammatory cytokine production, indicate that the pro-inflammatory response is decreased and physiologic function is improved in β2M/αAsGM1 mice subjected to CLP. The underlying mechanisms responsible for this improvement remain to be fully defined. However, it is likely that depletion of natural killer and CD8+ T cells, along with a reduction in pro-inflammatory cytokine production, contributes to better preserved hemodynamics and cardiac contractile function and the resistance of β2M/αAsGM1 mice to lethal intra-abdominal sepsis.

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References


Figure Legends

Figure 1: Initial mean arterial pressure in mice after cecal ligation and puncture. At 18 hours after cecal ligation and puncture or sham procedures, mice underwent tracheostomy and the left internal carotid artery was cannulated. Mean arterial pressure was measured immediately after arterial cannulation. ★p<0.05 compared to sham mice. ♦p<0.05 compared to wild type mice. N = 6–10 mice per group. Values are expressed as the mean ± SEM.

Figure 2: Systemic hemodynamics in mice after cecal ligation and puncture. Mice underwent tracheal intubation, carotid artery cannulation and left ventricular conductance catheter placement 18 hours after sham or CLP procedures. Mice were resuscitated to left ventricular end-diastolic volumes of 40-50 μl prior to measurement of hemodynamic variables. ★p<0.05 compared to sham mice. ♦p<0.05 compared to wild type mice. N = 6-10 mice per group. Values are expressed as the mean ± SEM.

Figure 3: Representative pressure-volume loops from mice following cecal ligation and puncture. A 1.4F conductance catheter was placed in the left ventricle of mice at 18 hours after CLP or sham procedures. Mice were resuscitated to left ventricular end-diastolic volumes of 40-50 μl. The pressure-volume tracings were obtained during transient occlusion of the inferior vena cava.
Figure 4: Changes in dp/dt$_{\text{max}}$ and dp/dt$_{\text{min}}$ during sepsis. Mice underwent sham or CLP procedures. The left ventricle was cannulated with a 1.4F conductance catheter and left ventricular pressure and volume measurements were performed. A) β2M/αAsGM1 mice exhibit improved dp/dt$_{\text{max}}$ and dp/dt$_{\text{min}}$ compared to wild type mice following CLP. These variables were determined in sham and septic mice in the steady state. B) The relationship of dp/dt$_{\text{max}}$ to left ventricular end diastolic volume in sham and septic mice during brief IVC occlusion. ★p < 0.05 compared to sham. ●p<0.05 compared to wild type mice. N = 6-10 mice per group. Values are expressed as the mean ± SEM.

Figure 5: β2M/αAsGM1 mice exhibit improved myocardial contractility compared to wild type mice following CLP. Left ventricular pressures and volumes were measured in mice using a 1.4F conductance catheter during occlusion of the inferior cava at 18 hours after CLP. The end systolic pressure-volume relationship, maximal elastance and preload-recruitable stroke work were determined. A) The left ventricular end-systolic pressure-volume relationship in sham and septic mice. The slopes of the pressure-volume curves obtained in mice from each group were numerically averaged. B) Maximal left ventricular elastance (E$_{\text{max}}$) in sham and septic mice. C) Preload-recruitable stroke work in sham and septic mice. ★p < 0.05 compared to sham. ●p<0.05 compared to wild type mice. N = 6-10 mice per group. Values are expressed as the mean ± SEM.
Figure 1

Mean Arterial Pressure (mmHg)

- Sham
- WT
- $\beta 2M/\alpha$ AsGM1

Significance indicators:
- *: Significant difference
- : Significant difference
Figure 2
Figure 3
Figure 4
Figure 5