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Myocardial Inflammatory Responses to Sepsis Complicated by Previous Burn Injury

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Abstract

Background: It is generally accepted that an initial injury such as burn trauma alters immune function such that a second insult increases the morbidity and mortality over that observed with each individual insult. We have shown previously that either burn trauma or sepsis promotes cardiomyocyte secretion of TNF- α and IL-1 β , cytokines that have been shown to produce myocardial contractile dysfunction. This study determined whether a previous burn injury (given eight days prior to sepsis) (1) provides a preconditioning phenomenon, decreasing inflammatory responses to a second insult or (2) exacerbates inflammatory response observed with either injury alone.

Methods: Anesthetized Sprague-Dawley rats were given either burn injury over 40% total body surface area, sepsis alone (intratracheal *S. pneumoniae*, 4×10^6 colony forming units) or sepsis eight days after burn; all rats received lactated Ringer's solution. Hearts harvested 24 h after onset of sepsis alone or sepsis plus eight-day burn were used to (1) isolate cardiomyocytes (collagenase) or (2) assess contractile function (Langendorff). Cardiomyocytes loaded with 2 μ g/mL Fura-2AM or sodium-binding benzofuran isophthalate were used to measure intracellular calcium and sodium concentrations (Nikon inverted microscope, GrooneyTM optics, InCyt Im2TM Fluorescence Imaging System). Additional cardiomyocytes were used to measure myocyte-secreted TNF α , IL-1, IL-6, IL-10 (pg/ml, ELISA).

Results: Either burn trauma alone or sepsis alone promoted TNF- α , IL-1 β , nitric oxide, IL-6 and IL-10 secretion by cardiomyocytes ($p < 0.05$). Producing aspiration-related pneumonia eight days postburn produced myocardial pro- and anti-inflammatory responses and increased myocyte Ca²⁺/Na⁺ concentrations to a significantly greater degree than the responses observed after either insult alone.

Conclusions: A previous burn injury alters myocardial inflammatory responses, predisposing the burn-injured subject to exaggerated inflammation, which correlates with greater myocardial dysfunction.

INTRODUCTION

IT IS GENERALLY ACCEPTED that an initial injury such as burn trauma alters immune function such that a second insult increases the morbidity and mortality over that observed with each individual insult [1-7]. Burn injury-related defects in cell-mediated immune responses have also been shown to vary with the time between the initial burn injury and the septic insult [4,8,9]. The immunosuppression that occurs after burn injury has been examined in both clinical and experimental settings, and impaired interleukin-2 (IL-2) production has been identified as a significant component of the compromised immune response with injury, disease, and aging [8,10,11].

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Our interest in burn injury complicated by sepsis arose from the finding that pneumonia-related sepsis was a frequent occurrence in the burn patient population in our teaching hospital (Parkland Memorial Hospital, Dallas, TX) and was often the attributable cause of death in this patient population. In addition, the incidence of nosocomial pneumonia has been described as approximately 50% in burn patients both with and without smoke inhalation injury [13], and another study of more than 1,000 burn patients found that pneumonia increased the predicted burn-related mortality regardless of whether inhalation injury was present [14]. Because of these observations, we developed a model of aspiration pneumonia-induced sepsis in Sprague-Dawley rats given a full thickness burn over 40% of the total body surface area (TBSA) [15]. Our previous studies have shown that sepsis in the presence of a previous burn injury impaired cardiac performance and exacerbated the myocardial contractile dysfunction that was characteristic of either burn alone or sepsis alone [15,16]. However, a limitation of that previous work was that pneumonia developed either 48 or 72 h after burn injury. A review of the burn patient population in our teaching hospital suggested that pneumonia-related sepsis is most likely to occur 7–10 days after admission to the burn unit. The effects of delayed sepsis after an initial burn injury on myocardial inflammation and myocardial contractile function have not been examined.

Previous studies of sequential injury have suggested that an initial injury (first hit) may provide preconditioning, decreasing morbidity and mortality associated with a second injury (second hit) [12]. However, Moss et al. suggested that an initial injury such as thermal trauma alters cell-mediated immunity, increasing susceptibility to infection. Furthermore, these authors showed a temporal correlation between impaired immune response and susceptibility to infection, corroborating that impaired immune responses vary in accordance with the time period postburn [8].

Therefore, the purpose of this study was to determine whether a previous burn injury, given eight days prior to sepsis, stimulates a preconditioning phenomenon (decreasing inflammatory responses to a second insult), or exacerbates the inflammatory response seen with either injury alone. Our rat model included intratracheal (IT) administration of *Streptococcus pneumoniae* on day seven after a burn injury over 40% of the TBSA; all animals were studied 24 h after bacterial challenge (on day eight postburn). We hypothesized that burn injury complicated by pneumonia-induced sepsis would produce greater myocardial inflammation than seen with either insult alone, predisposing the burn-injured subject to greater myocardial contractile dysfunction after a subsequent episode of sepsis.

MATERIALS AND METHODS

Experimental model

Adult Sprague Dawley male rats (320–350 g) obtained from Harlan Laboratories (Houston, Texas) were conditioned in-house for five to six days after arrival with commercial rat chow and tap water available at will. All studies performed in this study were reviewed and approved by The University of Texas Southwestern Medical Center's Institutional Review Board for the care and handling of laboratory animals, and conformed to all guidelines for animal care as outlined by the American Physiological Society and the National Institutes of Health.

Catheter placement and burn procedure

Rats were anesthetized lightly with isoflurane 18–20 h before the burn experiment, and polyethylene catheters (PE-50) were inserted into the carotid artery and external jugular vein. Catheters were filled with heparinized saline, exteriorized, and secured at the nape of the neck. Eighteen hours after catheter placement, animals were anesthetized deeply (isoflurane), secured in a constructed template device, and exposed through the aperture in the template to

immersion in 100°C water for 10 sec on the back and upper sides. Use of the template produced well-circumscribed burned areas, avoided injury to the abdominal organs, and accomplished consistent full-thickness dermal burns over 40% of the TBSA. Sham burn rats were subjected to identical preparation, except that they were immersed in room temperature water to serve as controls. Immediately after immersion, rats were returned to individual cages and fluid resuscitation was begun (lactated Ringer's solution, 4 mL/kg/% burn, with one half of the calculated volume given during the first 8 h postburn and the remaining volume given over the next 16 h thereafter). Additional lactated Ringer's solution (one half of the volume administered during the first 24 h postburn) was given daily for three days after burn injury. Buprenorphine (0.5 mg/kg) was given every 8 h during the postburn period. Burned rats did not display discomfort or pain, moved freely about the cage, and consumed food and water within 20 min after recovering from anesthesia. In the sham burn group, the external jugular vein was cannulated and lactated Ringer's was given to maintain catheter patency (0.2 mL/kg/h); sham burn rats also received an identical regimen of buprenorphine throughout the study period. Body temperature, respiratory rate, systemic blood pressure, and heart rate were measured.

Preparation of inoculum

Streptococcus pneumoniae type 3 was obtained from the American Type Culture Collection (ATCC 6303, Rockville, MD) in lyophilized form. Bacteria were reconstituted and then passed through the cerebrospinal fluid of rabbit to increase virulence; aliquots were prepared and stored at -80°C. Before each experiment, individual aliquots were thawed, inoculated into Muller Hinton broth with supplement C (Difco, Kansas City, MO), and incubated overnight at 37°C in the presence of 5% CO₂. The broth was then centrifuged and the resultant pellet was washed twice with sterile endotoxin-free phosphate buffered saline (PBS) to remove any impurities adherent to the bacteria. The bacteria were then resuspended in sterile endotoxin-free PBS, agitated, and then drawn up into sterile tuberculin syringes in 0.3-mL aliquots. Bacterial colony forming units (CFU) were determined by plating 100 µL of the bacterial suspension onto blood agar plates in serial dilutions and incubating the plates overnight at 37°C. The number of viable bacteria inoculated into animals in either the pneumonia alone group or in the burn plus pneumonia groups was approximately 4×10^6 CFU [15,17,18]. Preliminary studies showed that the transtracheal injection of non-viable bacteria ($N = 4$) or PBS ($N = 3$) produced no ill effects and no cardiac dysfunction [16].

Induction of aspiration pneumonia

Seven days after either burn or sham burn, animals were again anesthetized with isoflurane, placed supine, and the area over the trachea was prepped with 10% povidine-iodine solution; the trachea was identified and isolated via blunt dissection through a midline incision. An aliquot of either bacterial suspension (4×10^6 CFU/0.3 mL) or sterile endotoxin-free PBS was injected directly into the trachea using a 27-gauge needle; the incision was then closed with surgical staples. Animals were placed on a 30° incline, with the head up, to ensure that the injected fluid entered the lungs. Anesthesia, IT injection, and closure of the incision were completed within four to five min.

Experimental groups

Twenty-four hours after catheter placement, rats were randomly allocated to receive either burn or sham burn injury over 40% of the TBSA (Table 1). All burned animals received fluid resuscitation and analgesic as described above. Seven days after the initial burn, sham burns were divided into subgroups to receive either intratracheal vehicle (PBS, 0.3 mL) to produce a sham burn/sham sepsis group (Group 1) or were given intratracheal *S. pneumoniae* (4×10^6 CFU in 0.3 mL of PBS) to produce sepsis in the absence of burn injury (Group 2). Similarly, groups of burned rats were given either intratracheal vehicle to produce burn injury in the

absence of sepsis (Group 3) or IT *S. pneumoniae* (4×10^6 CFU in 0.3 mL of PBS) to produce sepsis complicated by a previous burn injury (Group 4). One group of burned rats ($N = 6$) was studied 24 h postburn in the absence of sepsis to confirm our previous reports of burn-related myocardial inflammation and contractile dysfunction [16]. All experimental groups were studied 24 h after intratracheal administration of *S. pneumoniae* (day eight postburn) (Table 1). The development of sepsis in our rat model was determined from histological evidence of pulmonary infection and inflammation and the presence of bacteremia [15,16].

Isolated coronary-perfused hearts

Twenty-four hours after IT challenge, rats from each experimental group ($N = 7-9$ rats/group) were heparinized, a blood sample was collected to measure circulating cytokine concentrations and to determine the presence/absence of circulating bacteria. One lung was harvested and placed in buffered formalin for examination by a veterinary pathologist who was unaware of the origin of tissue with regard to group assignment. The remaining lung was lavaged with 25 mL ice-cold (4°C) PBS and bronchoalveolar lavage (BAL) samples were used to determine the presence/absence of bacteria. Hearts were removed and placed in ice-cold Krebs-Henseleit bicarbonate buffered solution (in mM: 118 NaCl, 4.7 KCl, 21 NaHCO₃, 1.25 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 11 glucose; pH, 7.4; pO₂, 550 mm Hg; PaCO₂, 38 mm Hg). A 17-gauge cannula placed in the ascending aorta was used to maintain perfusion of the coronary arteries by retrograde perfusion of the aortic cannula. Contractile function was assessed by measuring intraventricular pressure with a balloon cannula threaded through the apex of the left ventricular chamber. Peak systolic left ventricular (LV) pressure and LV end-diastolic pressure were measured, and $\pm dP/dt$ were obtained using an electronic differentiator (Model 7P20C, Grass Instruments, Inc., Quincy, MA). Left ventricular developed pressure was calculated by subtracting end-diastolic pressure from peak systolic LV pressure. A Grass PolyVIEW Data Acquisition System was used to digitize acquired data [19,20].

Cardiomyocyte isolation

To isolate cardiomyocytes, rats ($N = 5-6$ per experimental group) received an intraperitoneal injection of heparin (2,000 U) 20–30 min before sacrifice. The rats were decapitated, hearts were harvested and placed in a Petri dish containing room-temperature heart medium (in mM: 113 NaCl; 4.7 KCl; 0.6 KH₂PO₄; 0.6 Na₂HPO₄; 1.2 MgSO₄; 12 NaHCO₃; 10 KHCO₃; 20 D-glucose; 10 Hepes; 30 taurine; 2.0 carnitine; and 2.0 creatine; $0.5 \times$ MEM (minimum essential medium) amino acids [50 $\times$, Gibco/BRL 11130-051] which was bubbled constantly with 95% O₂–5% CO₂. Hearts were cannulated via the aorta and perfused with heart medium (12 mL/min for 5 min). Enzymatic digestion was initiated by perfusing the heart with digestion solution (34.5 mL of heart medium plus 50 mg of collagenase II, 50 mg bovine serum albumin (BSA), Fraction V, 0.5 mL trypsin, 100 mM CaCl₂, and 40 mM BDM (2,3-butanedione monoxime)) at a flow rate of 12 mL/min for 20 minutes at 37°C. The ventricles were mechanically disassociated in 6 mL of enzymatic digestion solution containing a 6 mL aliquot of $2 \times$ BDM/BSA solution (3 mg BSA, Fraction V to 150 mL of BDM stock, 40 mM), and the tissue homogenate was filtered. The cells were washed with 10 mL of $1 \times$ BDM/BSA solution (prepared by combining 100 mL of BDM stock, 40 mM; 100 mL of heart medium described above, and 2 g of BSA, Fraction V), and pelleted for 10 min. The pellet was resuspended in 10 mL of $1 \times$ BDM/BSA; the cells were washed and pelleted further in BDM/BSA buffer with increasing increments of calcium (100 μ M, 200 μ M, 500 μ M, to a final concentration of 1000 μ M). The final pellet was resuspended in MEM (minimum essential medium prepared by adding 10.8 g $1 \times$ MEM, 1 g NaHCO₃, 2.3 g Hepes, and 10 mL penicillin/streptomycin (Gibco, Grand Island, NY); 100 $\times$ with 950 mL MilliQ water) (Hydro, Durham, NC); total volume was adjusted to 1 L (pH 7.1) [21].

Cytokine secretion by cardiomyocytes

Myocytes were pipetted into microtiter plates at 5×10^4 cell/mL/well (12-well cell culture cluster, Corning Inc., Corning, NY) for 18 h (CO_2 incubator at 37°C). Supernatants were collected to measure myocyte-secreted TNF- α , IL-1 β , IL-6, IL-10 and NO $^\cdot$ (rat ELISA, Endogen, Woburn, MA). Cell viability, cardiomyocyte cell number, and non-myocyte cell number were determined for each preparation; less than 2% of the total cell number in a myocyte preparation was non-cardiomyocytes regardless of experimental group. We examined previously the contribution of contaminating cells (non-myocytes) in our cardiomyocyte preparations using flow cytometry, cell staining (hematoxylin and eosin), and light microscopy; even a two- to threefold increase in the number of contaminating leukocytes in myocyte preparations could not account for the burn-related rise in myocardial cytokine secretion [20]. Thus, the inflammatory cytokines measured in the cardiomyocyte supernatant in this study were indeed cardiomyocyte-derived.

Intracellular calcium ($[\text{Ca}^{2+}]_i$) and sodium ($[\text{Na}^+]_i$) measurements

Separate aliquots of cells were loaded with either Fura-2 AM for 45 min or sodium-binding benzofuran isophthalate (SBFI) for 1 h at room temperature in the dark. Myocytes were then suspended in 1.0 mM calcium containing MEM, washed to remove extracellular dye; myocytes were placed on a glass slide on the stage of an inverted microscope (Nikon, Melville, NY). The microscope was interfaced with Grooney optics for epi-illumination, a triocular head, phase optics, 30 $\times$ phase contrast objective, and a mechanical stage. An excitation illumination source (300 W compact Xenon arc illuminator) was equipped with a power supply. In addition, this InCyt Im2TM Fluorescence Imaging System (Intracellular Imaging, Cincinnati, Ohio) included an imaging workstation and Intel Pentium Pro 200 MHz-based PC. The computer controlled filter changer allowed alternation between the 340- and 380-nm excitation wavelengths. Images were captured by monochrome charge-coupled device (CCD) camera equipped with a TV relay lens. InCyt Im2TM image software allowed measurement of intracellular calcium and sodium concentrations from the ratio of the two fluorescent signals generated from the two excitation wavelengths; background was removed by the software. The calibration procedure included measuring fluorescence ratio with buffers containing different concentrations of either calcium or sodium. At each wavelength, the fluorescence emissions were collected for 1-min intervals and the time between data collection intervals was 1–2 min. Since quiescent or non-contracting myocytes were used in these studies, the calcium concentrations measured reflected diastolic concentrations.

Statistical analysis

All values are expressed as mean values $\pm$ standard error of the mean. Analysis of variance (ANOVA) was used to assess an overall difference among the groups for each of the variables. Levene's test for equality of variance was used to suggest the multiple comparison procedure to be used. If equality of variance among the four groups was suggested, multiple comparison procedures were performed (Bonferroni); if inequality of variance was suggested by Levene's test, Tamhane multiple comparisons (which do not assume equal variance in each group) were performed. Probability values less than 0.05 were considered statistically significant (analysis was performed using SPSS for Windows, Version 7.5.1, SPSS, Inc., Chicago, IL).

RESULTS

Animals given sham burn injury and sham sepsis (Group 1) remained alert and healthy; there were no deaths among these animals during the experiments. After burn injury, animals moved freely about the cage and there was no evidence of burn-related pain. Histologic examination of lung tissue showed that *S. pneumoniae* challenge produced consistent evidence of pulmonary infection and inflammation that was severe but comparable in all animals given intratracheal

bacteria. Blood and bronchoalveolar cultures from rats with sepsis in the absence of burn injury (Group 2) or in burn complicated by sepsis (Group 4) were positive at 24 h after intratracheal administration of *S. pneumoniae*. Furthermore, bacterial counts (CFU/mL) of bronchoalveolar lavage fluid were similar in sepsis alone (6.8×10^{11}) and in burn plus sepsis (7×10^{11}); similarly, CFU/mL in the blood was not different in sepsis alone (7.4×10^{12}) and burn plus sepsis (8×10^{12}). Lungs from the sham burn/sham sepsis group (Group 1) and in rats with burn injury in the absence of sepsis (Group 3) were histologically normal; blood and BAL cultures from these groups were negative for *S. pneumoniae*.

Hemodynamic—metabolic responses to burn sepsis

At 24 h after burn injury in the absence of sepsis (Group 5), mean arterial blood pressure, serum ionized calcium concentration, hemoglobin concentration, and hematocrit were significantly lower than values measured in time-matched sham burns (Table 2). In addition, blood lactate concentrations and base excess rose significantly 24 h after burn injury alone. Eight days after burn trauma in the absence of sepsis (Group 2), serum calcium concentrations remained significantly lower than values measured in shams, whereas blood lactate concentrations remained above sham values. Sepsis in the absence of burn of injury was associated with mean arterial blood pressure and serum ionized calcium concentrations that were significantly lower than values measured in sham animals. In addition, sepsis produced significant metabolic acidosis as indicated by the elevated blood lactate concentration and the change in base excess. Sepsis complicated by a previous burn injury (Group 4) was associated with a lower mean arterial blood pressure and lower serum ionized calcium concentration compared to values measured in shams (Group 1). In addition, metabolic acidosis in sepsis complicated by previous burn injury was evident from the higher blood lactate and change in base excess compared to values measured in shams. Finally, sepsis that developed eight days after a previous burn injury was associated with a lower mean arterial blood pressure, lower heart rate, and lower serum ionized calcium concentration compared to values measured in sham.

Circulating cytokine levels after burn or burn plus sepsis

Twenty-four hours after burn injury in the absence of sepsis (Group 5, Table 3), plasma IL-1 β , IL-6, IL-10 and NO $^{\cdot}$ concentrations were significantly higher than values measured in sham burns (Group 1, $p < 0.05$). Eight days after burn injury in the absence of sepsis, IL-1 β , IL-6 and IL-10 values remained elevated above those measured in shams whereas plasma TNF- α values rose to levels that were significantly higher than values measured in shams (Group 2, $p < 0.05$). Sepsis alone (Group 3) produced a pro-inflammatory response as evidenced by the rise in plasma TNF- α , IL-1 β , and NO $^{\cdot}$ concentrations, whereas an anti-inflammatory response was evident from the rise in circulating IL-10 concentrations. Sepsis that occurred eight days after a previous burn injury (Group 4) greatly exaggerated both the anti- and pro-inflammatory responses compared to those observed with sepsis alone (Group 3, $p < 0.05$).

Cardiac contractile responses to burn and/or sepsis

As shown in Table 4, left ventricular developed pressure and $\pm$ dP/dt max were significantly reduced at 24 h after burn (Group 5) compared to time matched values measured in sham burns (Group 1). All indices of left ventricular performance improved after burn trauma, returning to values that were not significantly different than those measured in sham by eight days postburn (Group 2). Similarly, 24 h after intratracheal *S. pneumoniae* challenge, LVP and $\pm$ dP/dt max were significantly lower (Group 3) than values measured in sham and these data were similar to those measured 24 h after burn alone or 24 h after IT *S. pneumoniae* administration in animals with a previous burn injury (Group 4).

Left ventricular function curves were calculated for all experimental groups by plotting left ventricular developed pressure and $\pm$ dP/dt max versus incremental increases in preload or left

ventricular volume (Fig. 1). Either burn alone (24 h postburn) or sepsis alone impaired left ventricular contraction and relaxation as indicated by the downward shift of the left ventricular function curves calculated for these groups and compared to function calculated for the sham burn/sham sepsis group. Producing sepsis eight days after burn injury exacerbated the cardiac contractile dysfunction seen with sepsis alone, producing measures of LVP and $\pm dP/dt$ max that were significantly lower than values measured in sepsis alone ($p < 0.05$).

Myocardial inflammatory responses to burn injury and/or sepsis

At 24 h after burn injury, there was a significant increase in cardiomyocyte secretion of inflammatory mediators TNF- α and IL-1 β (Fig. 2), NO $^{\cdot}$ (Fig. 3), and anti-inflammatory cytokines IL-6 and IL-10 (Fig. 4). Eight days after burn trauma, TNF- α , IL-1 β , NO $^{\cdot}$, IL-6, and IL-10 secretion by cardiomyocytes remained significantly elevated over values measured in time-matched shams ($p < 0.05$). Sepsis in the absence of burn injury produced a significant rise in pro- and anti-inflammatory cytokine secretion by cardiomyocytes that was similar to that seen 24 h after burn alone. In contrast, sepsis that occurred eight days after a previous burn injury exacerbated TNF- α , IL-1 β , NO $^{\cdot}$, IL-6, and IL-10 secretion by cardiomyocytes compared to the cytokines secreted by cardiomyocytes prepared after sepsis alone ($p < 0.05$).

Cardiomyocyte sodium and calcium concentrations in burn and/or sepsis

Either burn injury (24 h postburn) or sepsis alone promoted significant cardiomyocyte calcium and sodium loading (Fig. 5) compared to sodium and calcium concentrations measured in cardiomyocytes prepared from hearts from sham burn/sham sepsis (Group 1). Eight days after burn injury, myocyte sodium and calcium concentrations fell from values measured 24 h after burn alone ($p < 0.05$) but remained above values measured in sham burns ($p < 0.05$). Sepsis, which occurred in the absence of burn trauma, promoted a rise in cardiomyocyte sodium and calcium concentrations. Whereas calcium accumulation by cardiomyocytes prepared from animals that developed sepsis eight days postburn tended to be higher than calcium concentrations measured in cardiomyocytes prepared from animals with sepsis alone, this difference was not significant. Therefore, calcium accumulation by cardiomyocytes was similar after either burn injury alone (24 h post-burn), sepsis alone, or sepsis complicated by burn injury eight days earlier. In contrast, cardiomyocyte sodium concentrations were significantly higher 24 h after burn alone or after sepsis that occurred eight days postburn, compared to sodium concentrations measured after sepsis alone ($p < 0.05$).

DISCUSSION

These data confirm that sepsis, eight days after a previous burn injury, exacerbated myocardial inflammation over that seen with either burn injury alone or sepsis alone. In addition, sepsis complicated by a previous burn injury exacerbated the myocardial contractile abnormalities that are characteristic of either injury alone [15,16,19-21]. These data are consistent with previous reports that an initial traumatic injury alters immunologic function, increasing susceptibility to infection and worsening outcome with a second challenge such as sepsis [22]. Several previous studies have shown that immune responses after burn injury and susceptibility to infection vary in direct proportion to the postburn time interval [4,8,9]. In this regard, splenocyte IL-2 responses to phytohemagglutinin are impaired after burn injury, and the extent of the cell-mediated immune defect varies with time lapse after thermal injury [8]. Furthermore, burn-related immune dys-function has been confirmed in experimental models of burn injury [22-26] as well as in clinical burns [4,9].

In our study, infectious challenge after burn trauma was accomplished by intratracheal administration of *S. pneumoniae*. This approach was based on previous findings that pneumonia is a frequent occurrence after burn injury with and without inhalation injury [13].

A standardized thermal injury produced well-circumscribed burns, uniform in depth, while avoiding injury to underlying organs. This injury model has been associated with robust cardiomyocyte inflammatory responses as well as profound myocardial contractile dysfunction [19-21]. In this present study, upregulation of pro-inflammatory as well as anti-inflammatory cytokine responses persisted over the eight days postburn while myocardial contractile function gradually return toward levels measured in sham burns. Similarly, intratracheal administration of *S. pneumoniae* produced well-defined lung inflammation and infection as well as positive blood cultures 24 h after IT bacterial challenge. The lung inflammation, bacteremia, and myocardial contractile abnormalities that developed after *S. pneumoniae* challenge closely mimic the postburn complications that develop in clinical patient populations, providing an excellent model of burn complicated by subsequent sepsis.

The greater myocardial inflammatory cytokine responses to sepsis complicated a previous burn injury correlated with the greater cardiac contractile dysfunction in this group. We considered that the greater cardiomyocyte inflammation in the sepsis or burn plus sepsis groups may have been related to a greater number of non-myocytes (perhaps leukocytes or macrophages) contaminating the myocyte preparations; however, there was no significant difference in myocyte number or in contaminating non-myocyte cells among the experimental groups. Thus, the cytokine concentrations measured in supernatants were primarily cardiomyocyte derived.

That inflammatory cytokines mediate myocardial contractile dysfunction has been suggested by previous studies which confirmed the negative inotropic effects of TNF- α or IL-1 β applied alone or in combination to isolated ventricular muscle preparations or isolated cardiomyocyte preparations [27,28]. Additional support for the cardiodepressive effects of inflammatory cytokines in injury and disease has been provided by reports that anti-TNF strategies provide significant cardioprotection in sepsis, burn injury, and trauma [29-31]. Whereas circulating proinflammatory and anti-inflammatory cytokine concentrations rose after burn injury alone, sepsis alone or burn complicated by sepsis, it is likely that local concentrations of cytokines generated within the myocardium play a primary role in burn- or sepsis-related myocardial contractile abnormalities. It is expected that myocardial concentrations of inflammatory cytokines are not rapidly buffered or neutralized by endogenous scavenging mechanisms [30]. In our model, IL-10 secretion by cardiomyocytes also rose after burn injury; these data contrast with studies by Napolitano et al. who described a significant decrease in IL-10 production by other cell types after burn injury that was paralleled by altered T-cell activation [32,33]. Differences in IL-10 responses measured in cardiomyocytes versus the splenocytes may be related to differences in the animal models or to differences in cell type selected for study.

The mechanisms by which either burn injury alone, sepsis alone, or sepsis complicated by previous injury promote cardiomyocyte synthesis and secretion of pro- and anti-inflammatory cytokines remains unclear. However, our previous studies have suggested that signaling through the Toll/IL-1 pathway plays a role in inflammatory responses to injury as well as to infection. Stimulation of the myocardial Toll/IL-1 pathway activates p38 MAP kinase, promoting I κ B phosphorylation and NF- κ B nuclear translocation, culminating in cardiomyocyte cytokine secretion [19,20]. While these mechanisms play a role in the cardiac contractile defects that are characteristic of either burn alone or infection alone, these intracellular signaling mechanisms likely play a role in myocardial inflammatory responses to sepsis that occurs after a previous burn injury.

While our studies confirm that sepsis that developed eight days after an initial burn injury produced significantly greater myocardial inflammation and greater myocardial contractile deficits than observed after either injury alone, the limitations of this rodent animal model are clearly recognized. It is frequently difficult to mimic the multitude of complications that occur

in critically ill burned patients, but our study did include the presence of devitalized tissue, fluid and electrolyte shifts, and aggressive fluid resuscitation. This model of pneumonia-related sepsis, designed to mimic a frequent cause of sepsis in the clinical arena, provides a model to explore several aspects of myocardial inflammation and dysfunction that occur in sepsis complicated by a previous injury. A better understanding of the complex pro-inflammatory and anti-inflammatory myocardial responses to sepsis complicated by previous injury may allow the development of appropriate therapeutic strategies designed specifically to control myocardial inflammation, provide cardioprotection, and improve outcome.

In summary, our studies confirm that pneumonia-related sepsis or major burn injury independently promoted myocardial inflammation and myocardial contractile abnormalities. Sepsis that occurred eight days after a previous burn injury produced significantly greater myocardial inflammation and greater myocardial contractile depression than responses observed 24 h after either burn injury alone or sepsis alone. Our data suggest that therapeutic strategies that would either decrease inflammatory responses to burn injury or provide cardiac support during the postburn period may decrease morbidity and improve outcome in burn patients who are at risk for developing sepsis.

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Table 1.
EXPERIMENTAL P_{ROTOCOL}.

FIG. 1.

Left ventricular developed pressure (LVP) and $\pm dP/dt$ max responses to incremental increases in left ventricular volume. Experimental groups include Group 1 (sham), Group 2 (8 days postburn), Group 3 (sepsis alone), Group 4 (sepsis which occurs eight days postburn), and Group 5 (24 h postburn). All values are $\pm$ SEM. *Significant difference from sham at $p < 0.05$.

FIG. 2. TNF- α (upper panel) and IL- β (lower panel) secretion by cardiomyocytes from all experimental groups. All values are $\pm$ SEM. *Significant difference from sham at $p < 0.05$. +Significant difference from sepsis alone at $p < 0.05$, unpaired t -test.

FIG. 3. Nitric oxide secretion by cardiomyocytes from all experimental groups. All values are $\pm$ SEM. *Significant difference from sham at $p < 0.05$. +Significant difference from sepsis alone at $p < 0.05$, unpaired t -test.

FIG. 4. IL-6 (upper panel) and IL-10 (lower panel) secretion by cardiomyocytes from all experimental groups. All values are $\pm$ SEM. *Significant difference from sham at $p < 0.05$. +Significant difference from sepsis alone at $p < 0.05$, unpaired t -test.

FIG. 5. Cardiomyocyte calcium concentrations (upper panel) and sodium concentrations (lower panel) in all experimental groups. All values are $\pm$ SEM. *Significant difference from sham at $p < 0.05$.

Table 2

HEMODYNAMIC AND METABOLIC RESPONSES TO BURN AND/OR SEPSIS

	Group 1 sham	Group 2 (8-day burn)	Group 3 (sepsis)	Group 4 (8-day burn 1 sepsis)	Group 5 (24-h burn)
MAP	151 ± 5	145 ± 4	129 ± 6 [*]	102 ± 5 ^{**}	112 ± 8 [*]
HR	523 ± 12	505 ± 21	497 ± 12	421 ± 29	500 ± 9
pH	7.47 ± 0.04	7.51 ± 0.01	7.49 ± 0.03	7.51 ± 0.2 [†]	7.48 ± 0.02
pCO ₂	24 ± 1	28 ± 1	25 ± 2	26 ± 1	26 ± 1
pO ₂	116 ± 5	108 ± 3	105 ± 5	104 ± 3	113 ± 4
Hemoglobin	14.0 ± 1.0	12.4 ± 0.5	13.3 ± 1.1	11.8 ± 0.5 [*]	10.5 ± 0.8 [*]
Hct	42.8 ± 3.0	37.3 ± 1.5	41.0 ± 3.3	35.6 ± 1.6 [†]	31.7 ± 2.5
HCO ₃	19.5 ± 0.7	22.4 ± 0.6 [*]	18.7 ± 0.8	20.1 ± 0.5	21.4 ± 1.1
O ₂ saturation	99.8 ± 0.2	99.6 ± 0.2	98.7 ± 0.4	98.8 ± 0.05	99.5 ± 0.2
O ₂ content	21.5 ± 1.2	16.4 ± 0.6 [*]	18.0 ± 1.4	16.8 ± 0.7 [*]	21.4 ± 0.9 [*]
Ca ⁺⁺	1.29 ± 0.01	0.82 ± 0.01 [*]	1.00 ± 0.04 [*]	0.85 ± 0.02 [†]	0.85 ± 0.04 [*]
T°C	38.8 ± 0.1	38.8 ± 0.1	38.6 ± 0.1	38.9 ± 0.1	38.8 ± 0.1 [*]
Lactate	2.20 ± 0.3	4.14 ± 0.31 [*]	4.6 ± 0.2 [*]	3.11 ± 0.22 [*]	3.34 ± 0.4 [*]
Na	141 ± 1	145 ± 1	144 ± 1	144 ± 1	138 ± 1
Base excess	1.64 ± 0.4	0.52 ± 0.58 [*]	-1.27 ± 0.9 [*]	-1.7 ± 0.3 [*]	-2.17 ± 0.68 [*]

All values are mean ± SEM.

MAP, mean arterial pressure in mm Hg; HR, heart rate in beats per minute; HB, hemoglobin in g %; Hct, hematocrit; HCO₃, serum bicarbonate in mEq/L; Ca²⁺, serum ionized calcium in mmol/L; T, body temperature in degree C; lactate in mmMol; Na⁺, sodium in mmol/L; base excess in mmMol.

^{*} Significant burn-related difference compared to the respective control group at $p < 0.05$.

[†] Significant difference compared to sepsis in the absence of burn injury at $p < 0.05$.

Table 3

PLASMA CYTOKINE CONCENTRATIONS AFTER BURN AND/OR SEPSIS

	Group 1 (sham)	Group 2 (8-day burn alone)	Group 3 (sepsis alone)	Group 4 8-day burn + sepsis	Group 5 (24-h burn alone)
TNF- α (pg/mL)	3.6 $\pm$ 0.3	9.3 $\pm$ 0.7 [*]	8.4 $\pm$ 0.9 [*]	22.6 $\pm$ 1 ^{*+}	4.8 $\pm$ 0.2
IL-1 β (pg/mL)	2.3 $\pm$ 0.1	10 $\pm$ 0.6	29 $\pm$ 3 [*]	40 $\pm$ 2 ^{*+}	8.3 $\pm$ 0.6 [*]
IL-6 (pg/mL)	60 $\pm$ 4	262 $\pm$ 28 [*]	681 $\pm$ 12 [*]	1,241 $\pm$ 53 ^{*+}	249 $\pm$ 32 [*]
IL-10 (pg/mL)	4 $\pm$ 0.3	21.6 $\pm$ 1.5 [*]	89 $\pm$ 3 [*]	290 $\pm$ 4 ⁺	10 $\pm$ 0.3 [*]
Nitric oxide (μ /mL)	3.0 $\pm$ 0.3	4.3 $\pm$ 0.15	9.2 $\pm$ 0.2 [*]	16.7 $\pm$ 0.3 ^{*+}	7.6 $\pm$ 0.8 [*]

All values are mean $\pm$ SEM.^{*} Significant burn-related difference compared to the sham group at $p < 0.05$.⁺ Significant difference between sepsis alone and sepsis complicated by a previous burn injury at $p < 0.05$.

Table 4

CARDIODYNAMIC RESPONSES TO BURN, SEPSIS, OR BURN PLUS SEPSIS

	Group 1 (sham) (N = 6)	Group 2 (8-day burn) (N = 6)	Group 3 (24-h sepsis) (N = 5)	Group 4 (8-day burn & 24-h sepsis) (N = 5)	Group 5 (24-h burn) (N = 8)
LVP (mm Hg)	93 ± 5	85 ± 2	58 ± 3*	65 ± 4*	63 ± 4*
+dP/dt max (mm Hg/sec)	2021 ± 122	1965 ± 45	1250 ± 97*	1320 ± 131*	1309 ± 59*
dP/dt max (mm Hg/sec)	1725 ± 126	1710 ± 49	954 ± 79*	1180 ± 143*	1025 ± 98*
-dP40 (mm Hg/sec)	1793 ± 122	1775 ± 44	1184 ± 53*	1140 ± 116*	1249 ± 1108*
TPP (msec)	85.7 ± 0.9	84.7 ± 2.6	90.5 ± 1.7	91.4 ± 5.8	88.0 ± 2.9
RT90 (msec)	83.7 ± 1.9	79.3 ± 2.1	84.7 ± 1.6	88.0 ± 6.0	82.9 ± 2.0
Time to Max +dP/dt (msec)	56.3 ± 0.9	56.7 ± 0.9	53.3 ± 1.0	52.6 ± 1.7	49.1 ± 1.2
Time to Max -dP/dt (msec)	56.8 ± 0.8	55.2 ± 5.0	51.1 ± 0.8	51.6 ± 2.1	50.3 ± 0.5
CPP (mm Hg)	55.0 ± 2.7	51.8 ± 5.0	47.4 ± 2.6	44.2 ± 5.9	52.9 ± 6.6
CVR (mm Hg)	11.0 ± 0.6	10.4 ± 1.0	9.5 ± 0.5	8.8 ± 1.2	10.6 ± 1.32
HR (bpm)	262 ± 15	265 ± 4	261 ± 7	257 ± 14	253 ± 13

All values are mean ± SEM.

LVP, left ventricular pressure; ±dP/dt max in mm Hg/sec; dP40, left ventricular developed pressure at 40 mm Hg; TPP, time to peak pressure; RT90, time to 90% relaxation of ventricle; CPP, coronary perfusion pressure; CVR, coronary vascular resistance; HR, heart rate in beats per minute.

* Significant burn-related difference compared to the sham group at $p < 0.05$