Early infection during burn-induced inflammatory response results in increased mortality and p38-mediated neutrophil dysfunction

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Adediran SG, Dauplaise DJ, Kasten KR, Tschöp J, Dattilo J, Goetzman HS, England LG, Cave CM, Robinson CT, Caldwell CC. Early infection during burn-induced inflammatory response results in increased mortality and p38-mediated neutrophil dysfunction. Am J Physiol Regul Integr Comp Physiol 299: R918–R925, 2010. — Following burn injury, the host is susceptible to bacterial infections normally cleared by healthy patients. We hypothesized that during the systemic immune response that follows scald injury, the host’s altered immune status increases infection susceptibility. Using a murine model of scald injury under induced anesthesia followed by intraperitoneal infection, we observed increased neutrophil numbers and function at postburn day (PBD) 1 compared with sham-burned and PBD4 mice. Further, increased mortality, bacteremia, and serum IL-6 were observed in PBD1 mice after *Pseudomonas aeruginosa* (PA) infection compared with sham-burned and PBD4 mice infected with PA. To examine these disparate responses, we investigated neutrophils isolated at 5 and 24 h following PA infection from PBD1 and sham-burned mice. Five hours after infection, there was no significant difference in number of recruited neutrophils; however, neutrophils from injured mice had decreased activation, active-p38, and oxidative burst compared with sham-burned mice. In direct contrast, 24 h after infection, we observed increased numbers, active-p38, and oxidative burst of neutrophils from PBD1 mice. Finally, we demonstrated that in neutrophils isolated from PBD1 mice, the observed increase in oxidative burst was p38 dependent. Altogether, neutrophil activation and function from thermally injured mice are initially delayed and later exacerbated by a p38-dependent mechanism. This mechanism is likely key to the observed increase in bacterial load and mortality of PBD1 mice infected with PA.

Trauma; immune status; oxidative burst; murine

**BURN AND BLUNT TRAUMA INJURIES** induce whole-body inflammation classically defined as the systemic inflammatory response syndrome (SIRS). During SIRS, increased numbers of peripheral leukocytes become hyperactive and produce immunoregulatory cytokines such as TNF-α, IL-1, and IL-6 (1, 14, 42). Tissue damage from inflammation may involve vascular endothelial compromise, interstitial edema, coagulation imbalances, and production of reactive oxygen species and free radicals (20, 31–33). In burn patients, the combination of tissue injury, sustained inflammatory response, and loss of adaptive immune cells predisposes to infectious complications. Infections in thermally injured patients are often associated with opportunistic organisms such as *Pseudomonas aeruginosa* (PA), *Staphylococcus epidermidis*, and *Staphylococcus aureus* (26, 27, 41). Studies suggest that blunted bacterial clearance allows for organism propagation in the burn-injured host (12), possibly due to alterations in neutrophil and macrophage production of reactive oxygen species (6, 24).

The concurrent inflammatory and anti-inflammatory responses after trauma increase the risk of postinjury complications, mostly through nosocomial infection, sepsis, and multiple organ system failure (2, 28, 36). In the innate arm of the immune system, the inflammatory response involves increased neutrophil recruitment, activation, and functionality (19). Increased early peripheral leukocytosis with a subsequent decline to below sham levels has been demonstrated in a murine model of burn injury (4). Functional changes in neutrophils are influenced by downstream effects on NF-κB by mitogen-activated protein kinases (MAPK) such as p38, ERK, and AKT. Burn injury-induced priming of neutrophils was shown to be p38 dependent (6), while ERK activation during thermal injury was associated with enhanced neutrophil O2 production (13). Additionally, decreased neutrophil apoptosis and increased oxidative burst is associated with elevated levels of activated AKT (7, 17). Taken together, these reports suggest traumatic burn injury produces a hyperinflammatory innate immune response, yet the role of this response in infection has yet to be elucidated.

Previous reports in a scald burn model demonstrate that T-cell numbers and function are decreased 1 day following injury, while the innate and adaptive immune systems express a proinflammatory phenotype at 8 days following injury (44). These studies suggest that divergent immune responses occur following traumatic injury, with host response to subsequent infectious exposure based on immune status at the time of challenge. In support of this, it was recently shown that scalded mice challenged with *Escherichia coli* 7 days after a scald burn were less susceptible to infection (22). Taken together, we hypothesized that following scald burn injury, temporal immunological changes occur such that susceptibility to bacterial infection and tissue damage is altered. To address this hypothesis in a clinically relevant burn model, we first determined changes in immunological phenotype at multiple time points following a “first hit” thermal injury. Next, we subjected scald-injured mice to a “second hit” bacterial infection using a commonly encountered opportunistic organism and determined alterations in the immunological response. Our results clarify some of the mechanisms underlying neutrophil changes that occur during the innate immune response to burn trauma.

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**MATERIALS AND METHODS**

**Scald burn injury.** Male home-bred mice of the CF-1 outbred strain between 6 and 8 wk old (26–36 g) were used for all experiments. All experiments involving animals were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. Scald burn was inflicted as previously described (44). Briefly, normal-fed mice were anesthetized with 3% isoflurane, and hair was clipped from their dorsal surface. The mice were placed in a template exposing 18% of their dorsal surface, then immersed in a 90°C water bath for 9 s, producing a well-demarcated, full-thickness injury. Immediately following scald burn, mice were resuscitated with 1.7 ml of sterile saline intraperitoneally without postinjury analgesics. The mice were then allowed to recover for 3 h on a 42°C heating pad with ad libitum access to water. Sham-treated mice underwent similar anesthetic and hair clipping, were immersed in room temperature water, and then resuscitated with 1.7 ml of sterile saline intraperitoneally. Experiments were then completed on the basis of the study design demonstrated in Fig. 1.

**Intraperitoneal bacterial challenge.** For induction of systemic infection, mice were injected intraperitoneally on the indicated postinjury days (postburn day 1 or 4, PBD1 or PBD4) with 1 × 10⁶ colony-forming units (CFU) *Pseudomonas aeruginosa* (PA) (strain PA01) as previously described (18, 22) and demonstrated in Fig 1. Two-hundred microliters of stock PA01 was placed into 5 ml of PBS and incubated for 18 h overnight at 37°C. Solution was read at 600 optical density to confirm concentration of 1 × 10⁶ CFU prior to injection. Bacteria were plated on tryptic soy agar plates at relevant dilutions to verify concentration. Of note, PBD1 mice infected intraperitoneally with PA demonstrated clear signs of systemic infection, including rapid breathing, hunched posture, and pilorectation. These symptoms were not observed in sham mice, and to a lesser extent in PBD4 mice, infected with PA.

**Bacterial load determination.** Bacterial counts were performed on blood harvested aseptically by cardiac puncture as previously described (43). Samples were serially diluted in sterile saline and cultured on tryptic soy agar plates. Plates were incubated at 37°C for 24 h, and colony enumeration was performed.

**Peritoneal lavage harvest of neutrophils.** Peritoneal fluid was harvested from mice by lavage after aseptic preparation of the abdominal wall, injection of 9 ml of 0.9% normal saline intraperitoneally, gently agitation of the abdomen, and aspiration of lavage by syringe (43). Peritoneal lavage was dispensed into 15-ml conical tubes and placed on ice. Cells were subsequently utilized for flow cytometry and functionality assays.

**Cytokine measurement by ELISA.** Whole blood was harvested by sterile cardiac punch, placed into serum separator tubes, and centrifuged at 10,000 rpm for 10 min with subsequent into Eppendorf tubes. Serum IL-6 levels were analyzed by ELISA, according to the manufacturers’ protocols (Pepro Tech, Rocky Hill, NJ, and BioSource, Camarillo, CA).

**Flow cytometry for surface and intracellular staining.** Analyses of cell surface antigen expression and MAPK expression in situ were performed as previously described on peripheral blood, peritoneal lavage, and spleen samples (3, 38). Cells were resuspended in FACS buffer (PBS with 1% BSA and 0.1% azide). Nonspecific binding was controlled by adding 5% rat serum (Invitrogen, Carlsbad, CA) and 1 µg/tube Fe block (BD Biosciences, San Jose, CA) to the FACS buffer. Myeloid cells were surface stained with the following antibodies: CD11b (clone M1/70.15, BD Biosciences), 7/4 (clone 7/4, Invitrogen), Ly-6G (clone 1A8, BD Biosciences). Analysis of phospho-p38 (clone 28B10, T180/Y182, Cell Signaling, Beverly, MA), phospho-ERK (clone pT202/pY204, BD Biosciences), and phospho-AKT (clone D9E/Ser473, Cell Signaling) was performed on neutrophils obtained from the peritoneum at indicated time points following injury and infection. The gating strategy for identification of neutrophils in this study was as previously described (43, 44). Briefly, the size and granularity parameters were evaluated in a dot plot to identify appropriate cells. These cells were subsequently analyzed in a dot plot using 7/4 and Gr-1 expression to identify neutrophils. This population of neutrophils in this study was as previously described (43, 44). Briefly, the size and granularity parameters were evaluated in a dot plot to identify appropriate cells. These cells were subsequently analyzed in a dot plot using 7/4 and Gr-1 expression to identify neutrophils. This population of neutrophils in this study was as previously described (43, 44). Briefly, the size and granularity parameters were evaluated in a dot plot to identify appropriate cells. These cells were subsequently analyzed in a dot plot using 7/4 and Gr-1 expression to identify neutrophils. This population of neutrophils in this study was as previously described (43, 44). Briefly, the size and granularity parameters were evaluated in a dot plot to identify appropriate cells. These cells were subsequently analyzed in a dot plot using 7/4 and Gr-1 expression to identify neutrophils. This population of neutrophils in this study was as previously described (43, 44). Briefly, the size and granularity parameters were evaluated in a dot plot to identify appropriate cells. These cells were subsequently analyzed in a dot plot using 7/4 and Gr-1 expression to identify neutrophils. This population of neutrophils in this study was as previously described (43, 44).

**Flow cytometry oxidative burst.** Oxidative burst was determined as previously described (35). Polystyrene tissue culture dishes were coated with 1 µg/well fibronectin for 2 h at 37°C and 5% CO₂ and then washed. One hundred microliters of reaction mixture [10 mM sco peptide, 1 mg/ml horseradish peroxidase, 4 mM NaN₃ in Kreb’s Ringer’s phosphate glucose buffer (KRPG)] and 20 µl neutrophils resuspended in KRPG at 7.5 × 10⁵ cells/ml were allowed to incubate at room temperature for 2 h at 37°C and 5% CO₂.

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**Fig. 1.** Timeline representation of one- and two-hit injury model. A: time after sham or scald injury that post-burn day (PBD) 1 and PBD4 mice were killed. B: time after sham or scald injury that PBD1 and PBD4 mice were injected with PA and then killed. C: time after sham or scald injury that PBD1 mice were injected with PA and then killed.
10 min at 37°C in the wells prior to stimulation with either buffer or formyl-methionyl-leucyl-phenylalanine (fMLP) (100 nM). Fluorescence was measured immediately and at 10-min intervals for 60 min. H$_2$O$_2$ production was calculated from the decrease in fluorescence due to oxidation of scopoletin. The data are expressed as nanomoles of H$_2$O$_2$ produced per 1.5 $\times$ 10$^4$ polymorphonuclear neutrophils (PMNs). For p38 inhibition experiments, neutrophils were first treated with 10 $\mu$M p38 inhibitor SB203580 prior to oxidative burst measurement.

Statistics. Statistical comparisons were performed using Kaplan-Meier LogRank (survival), Student’s t-test (two groups), or one-way ANOVA with Holm-Sidak post hoc test (more than two groups) using StatView 5.3 (SAS Institute, Cary, NC) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). The mean and standard error of the mean were calculated in experiments containing multiple data points. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Immunological phenotype following thermal injury. Following trauma, the number and functionality of leukocytes are altered (19). The injury type, severity, and host genetic predisposition are key to changes in the immune status. Here, we sought to determine the immune phenotype in mice after burn using flow cytometry. We observed a two-fold increase in circulating neutrophils 1 day following injury, while 4 days following injury, there was no significant difference compared with sham-burned mice (Fig. 2A). Additionally, neutrophils isolated from the PBD1 mice had a significantly increased fMLP-induced oxidative burst compared with neutrophils isolated from sham and PBD4 mice (Fig. 2B). Thus, 1 day following burn, the immune status shows increased neutrophil numbers and function compared with sham and PBD4 animals.

Survival, bacterial load, and IL-6 in two-hit trauma-infection model. After trauma, the injured host is more susceptible to an infection that would normally be cleared by a healthy host. To explore whether the observed changes in the immunological phenotype altered the response to an infection, we subjected sham, PBD1, and PBD4 mice to an intraperitoneal injection of 10$^6$ CFU of PA. Survival was then monitored over a 10-day period. This dose was chosen as healthy mice clear this dosage of inoculant with no noticeable side effects (data not shown). We observed that PBD1 mice had a significantly higher mortality compared with sham-burned and PBD4 mice (Fig. 3A). Next, we determined the systemic bacterial load 24 h following infection and observed that bacterial load in PBD1 mice was increased by at least an order of magnitude compared with sham-burned and PBD4 mice (Fig. 3B). In control experiments, we did not observe bacteremia in sham, PBD1, or PBD4 mice following thermal injury without a subsequent bacterial injection (data not shown). As the level of IL-6 in the circulation 6 h after an infection is known to be predictive of outcome (29), serum from control, PBD1, and PBD4 mice was measured for IL-6 at this time point following PA infection. We observed that serum IL-6 levels were ~6-fold higher in the uninfected PBD1 mice compared with the infected sham-burned and PBD4 mice. Evaluation of serum IL-6 from infected sham, PBD1, and PBD4 scald-burned mice again demonstrated no difference between sham and PBD4 animals. However, there was an approximately five-fold further increase in serum IL-6 levels of PBD1 mice infected with PA, significantly higher than the other two groups (Fig. 3C). Thus, these data show that the PBD1 mice had significantly increased mortality, systemic bacterial spread, and IL-6 following infection.

Neutrophil activity at the site of infection. As our previous results have showed that mortality and bacterial load were significantly different between healthy and PBD1 mice following PA infection, we focused on peritoneal neutrophil recruitment, activation, and function from these two groups. Five hours after bacterial challenge, peritoneal neutrophils were isolated from sham-burned and PBD1 mice. First, peritoneal neutrophil numbers were determined. Neutrophil recruitment to the site of infection was not significantly different between sham-burned and PBD1 mice (Fig. 4A) at 5 h postinfection. Neutrophils isolated from infected sham burned mice showed increased expression of CD11b at 5 h postinfection compared with those isolated from PBD1 mice (Fig. 4B). Next, we conducted intracellular staining of peritoneal neutrophils to evaluate signaling in situ. Here, we determined that neutrophils isolated from sham-burned mice had significantly increased active p38 and Akt expression compared with neutrophils isolated from PBD1 mice (Fig. 4C). We observed no significant differences in active ERK between these two groups of neutrophils (data not shown). Finally, upon fMLP treatment, we observed that neutrophils isolated from sham-burned mice had an approximate twofold increase in oxidative burst (Fig. 4D). Altogether, neutrophils isolated from infected, uninjured mice showed increased activation, signaling, and functionality.

Fig. 2. Altered neutrophil numbers and functionality in PBD1 mice compared with sham-burned and PBD4 mice are observed. Mice were subjected to scald injury as noted in MATERIALS AND METHODS and killed at postburn days 1 and 4. A: Peripheral blood neutrophil numbers determined using flow cytometry. B: oxidative burst was determined as described in MATERIALS AND METHODS. The sample size equals 5 to 13 per group, and values represent the average ± SE. *$P < 0.05$. **$P < 0.01$, compared with sham.
compared with infected PBD1 mice. Thus, healthy mice mounted a more robust immune response early after infection.

To further assess the response to a bacterial challenge, peritoneal lavage was conducted on uninjured and PBD1 mice 24 h after infection. We determined that neutrophil recruitment to the site of infection was increased 4.9-fold in PBD1 mice compared with control mice (Fig. 4A). The neutrophils isolated from infected PBD1 mice and sham-burned mice had increased CD11b expression compared with the 5-h time point. However, at the 24-h time point, we did not observe significant differences between the two groups (Fig. 4B). Intracellular staining of peritoneal neutrophils to evaluate active p38, ERK, and Akt demonstrated that neutrophils isolated from infected PBD1 mice had a 60% increase in active p38 (Fig. 4C), while there were no significant differences in active ERK (data not shown) and Akt between the two groups. Finally, upon IMLP treatment, we observed that neutrophils isolated from infected PBD1 mice had a 60% increase in active p38 (Fig. 4C), while there were no significant differences in active ERK (data not shown) and Akt between the two groups. Finally, upon IMLP treatment, we observed that neutrophils isolated from infected PBD1 mice had a 60% increase in active p38 (Fig. 4C), while there were no significant differences in active ERK (data not shown) and Akt between the two groups. Finally, upon IMLP treatment, we observed that neutrophils isolated from infected PBD1 mice had a 60% increase in active p38 (Fig. 4C), while there were no significant differences in active ERK (data not shown) and Akt between the two groups.

Neutrophil p38 inhibition decreases oxidative burst. We observed that neutrophils isolated from PBD1 mice 24 h after infection had increased active p38, as well as increased oxidative burst. To directly assess whether the increased expression of active p38 was associated with altered oxidative burst, we isolated neutrophils 24 h after infection and determined the oxidative burst with and without the p38 inhibitor, SB203580. Fig. 5A is a representative time course demonstrating a 2.5-fold increase in the oxidative burst of neutrophils isolated from infected PBD1 mice compared with sham-burned mice. Upon the addition of the p38 inhibitor, the oxidative burst of both groups is reduced to approximately the same level. In Fig. 5B, statistical analysis of multiple sample groups demonstrate that p38 inhibition results in a 75% vs. 45% oxidative burst reduction in neutrophils from infected PBD1 and sham mice, respectively. Thus, the increase in the oxidative burst of neutrophils isolated from infected PBD1 mice compared with sham-burned mice is mediated by p38.

Fig. 4. Increased mortality, bacteremia, and serum IL-6 in PBD1 mice compared with sham-burned and PBD4 mice after Pseudomonas aeruginosa (PA) infection. A: sham (n = 24), PBD1 (n = 20), and PBD4 (n = 14) mice were injected with PA intraperitoneally at specified times after scald burn and monitored for 10-day survival. The survival curve combines data from two independent experiments. B: whole blood bacterial CFU 24 h following bacterial injection, represented in logarithmic units. C: serum IL-6 levels 24 h after bacterial injection. The sample size equals four to eight per group and values represent the average ± SE. *P < 0.05, ***P < 0.001, compared with sham. #P < 0.05, compared with PBD1 scald only group. CFU, colony forming unit.
DISCUSSION

In evaluation of the innate response to burn, we demonstrate increased neutrophil numbers at the peritoneum, consistent with findings of delayed neutrophil apoptosis after traumatic injury (17). Additionally, neutrophils display augmented functionality through enhanced oxidative burst. The combination of elevated neutrophil numbers, oxidative burst, and delayed apoptosis typifies the proinflammatory immune response seen early postburn (6, 7, 17). Interestingly, 4 days following burn injury, the numbers and functionality of neutrophils are similar to sham animals. A report of decreased neutrophil oxidative burst and phagocytosis at 48 h following a 6% TBSA burn induced by hot blower suggests an oscillatory pattern of neutrophil functionality (4). The functionality of neutrophils following burn injury likely depends upon the mechanism of injury, time period following injury, and the tissue compartment from which these cells are collected. Here, our findings demonstrate that early after isolated burn injury, a robust response from increased numbers of overactive neutrophils occurs.

The phenotypic findings that we observed in scalded mice on PBD1 were similar to the divergent and concurrent immune response seen in blunt trauma patients (19). After establishing a clinically relevant model, we hypothesized that the adverse response to infection in burn mice was related to temporal immunological changes. We observed three distinct immune phenotypes in scald burn mice and evaluated their response to PA infection. The examined phenotypes included a sham burn mouse given intraperitoneal PA, a scald burn mouse infected 24 h after burn injury, and a scald burn mouse infected 4 days after burn injury. As shown in Fig. 3, sham and PBD4 mice infected with PA had similar survival, bacteremia, and serum IL-6 levels. In direct contrast, increased mortality, bacterial load, and serum IL-6 levels were found in the PBD1-infected mice. These data correlate with a flame burn model where substantially less subcutaneous PA was required in PBD1 mice.
to induce mortality compared with sham mice (37). Additionally, when PA was injected 3 days after burn injury, survival was similar between flame burn and sham mice, suggesting susceptibility to infection following burn is temporally related (37). Similarly, mice infected with *E. coli* soon after burn fared worse than sham mice, while survival of mice infected 3 to 4 days following injury was similar to sham animals (22). Our data suggest that as numbers and functionality of neutrophils return to sham levels following burn injury, survival and serum IL-6 levels in response to PA infection return to sham levels.

Interestingly, bacterial clearance is enhanced in PBD4 mice infected with PA, suggesting that priming of the innate response to infection occurs following burn injury. Indeed, damage-associated molecular patterns (DAMPs) are released during aseptic injury and serve to activate innate immunity (21). The repertoire of DAMPs is tissue specific and includes heat shock proteins, uric acid, and high-mobility group box 1 (47). It has been suggested that interaction between pattern-associated molecular patterns, of which LPS is a well-known example, and DAMPs results in the sepsis-like response following traumatic injury (47, 48). Recently, it was shown that mitochondrial DAMPs (MTDs) are released following muscular injury and activate p38 and ERK on neutrophils (48). Additionally, MTDs induced release of MMP-8 and IL-8 (48), both instrumental in PMN chemotaxis following injury (45). These reports implicate endogenous molecules in the involvement of the innate system priming that we demonstrate in this murine model of scald injury. Interestingly, while endogenous priming from tissue injury appears detrimental to infection clearance early, it provides a noted benefit later in the course of injury. Thus, temporal changes in the innate immune system following scald injury mediate the host response to subsequent infection.

On the basis of our findings, early events in the immune response to burn injury likely mediate outcomes following subsequent infection. As such, we investigated the innate immune response at 5 and 24 h following PA injection in PBD1 mice. Here, we observed a disparate response in mice at these time points. An increase in neutrophil recruitment, p-p38 signaling, and oxidative burst was seen 24 h after infection. In direct contrast, we showed similar neutrophil recruitment and decreased activation 5 h after infection in PBD1 mice. Additionally, p-p38 and p-AKT signaling was decreased, consistent with attenuated neutrophil function at the site of infection.

While these declines of 20% in p-p38 and p-AKT appear modest, they are in unison with our results of diminished neutrophil oxidative burst. We conclude that in PBD1-infected mice, the neutrophil response to bacteria is initially blunted due to changes in p-p38 and p-AKT signaling. This blunted neutrophil phenotype precludes adequate clearance of pathogen and results in further bacterial spread. With inadequate clearance of PA 5 h after infection in PBD1 mice, neutrophil recruitment and oxidative burst increase by 24 h after infection in an attempt to control the pathogen. As excessive oxidative burst involves the release of cytotoxic molecules, bystander tissue damage results and may lead to multiple system organ failure and mortality (15, 16, 34). Thus, a blunted innate response to infection early after scald burn precedes a hyperactive innate response in an attempt to control the pathogen.

We observed that peritoneal neutrophils in injured infected mice demonstrated increased active p38 levels 24 h after infection. Previous studies in thermally injured mice demonstrate significant systemic increases in TNF-α at 6 h postburn and IL-1β levels 12 h postburn (11, 25). These cytokines are known to prime neutrophils by increasing phosphorylation of p38 (6, 40). Here, we observed that neutrophils isolated from injured, infected mice had significantly increased oxidative burst 24 h after infection compared with neutrophils isolated from noninjured infected mice. This change in neutrophil functionality is positively correlated with percent expression of active p38 on neutrophils, consistent with a similar observation in trauma patients (8). To test the correlation that increased oxidative burst was associated with increased active p38, we exogenously added p38 inhibitor to peritoneal neutrophils isolated 24 h after PA infection. This inhibition of p38 reduced the oxidative burst back to the levels of neutrophils isolated from noninjured mice, verifying the association between active p38 and neutrophil oxidative burst. We speculate that phosphorylation of p47phox by active p38 in neutrophils may be responsible for the increase in fMLP-induced oxidative burst (9, 10). As fMLP-induced oxidative burst was not significantly elevated in thermally injured IL-1R1 knockout mice (5), we hope to complete future experiments to elucidate the role of...
this cytokine or possibly other mediators involved in neutrophil p38 phosphorylation in injured or septic mice.

Although this study demonstrates new insights into the response of neutrophils to scald injury and subsequent infection, it has limitations. The use of blood products, vasopressors, and ventilatory support in human burn patients influences and extends the systemic inflammatory response, an effect not easily replicated in mice. Additionally, restricted resuscitation and support therapies in mice limit the severity of burn injury that can be inflicted. However, our “first hit” results are consistent with numbers and functionality changes seen in human blunt trauma patients (19). We did not use analgesia after inducing a scald burn injury in these mice, consistent with our previous work (44). Our justification comes from extensive literature demonstrating reduction in neutrophil chemotaxis, oxidative burst, phagocytic function, and outcome in sepsis following opioid treatment in mice (46). The role of pain in postscaald burn inflammation remains to be fully elucidated in light of findings that neutrophils and macrophages release endogenous opioids during inflammatory states (30). Lastly, the purpose of this study was to determine molecular and mechanistic insights into why thermally injured patients are more susceptible to infections compared with healthy individuals. Although no single two-hit injury model using mice can mimic the human condition, this report utilized thermal injury followed by intraperitoneal injection of bacteria as previously described (18, 23, 39). This route of infection contrasts with other 2-hit thermal injury models that evaluate local (topical or subcutaneous) and organ-specific effects (intranasal) routes of infection, rapidly inducing bacteremia. We postulate that the systemic spread of bacteria in this model occurs due to delayed pathogen control at the site of infection, as is similarly observed with trauma or immune compromised patients in the intensive care unit. Thus, we speculate that the results from this report can be used to identify potential therapeutic targets to beneficially alter the immune response to infection following burn injury.

**Perspectives and Significance**

This study provides evidence that the innate response to infection following burn trauma fluctuates in a temporal fashion, with pathogen clearance mediated by the timing of infection after injury. The finding that neutrophil oxidative burst is at least partially mediated by p38 is important as a mechanistic target for future therapeutic intervention. Overall, our findings suggest the response to infection following burn injury is mediated by host immune status at the time of infection, suggesting a need for rapid and consistent immune status determination following traumatic injury. Future areas of study may include characterization of the temporal innate immune response to scald-induced inflammation and infection in a human burn patient population and development of a flow cytometric-based panel for determining immune status following traumatic injury. The results of these endeavors may enhance not only sepsis management following burns, but management in all septic patients as well.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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**GRANTS**

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