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Attenuating Burn Wound Inflammatory Signaling Reduces Systemic Inflammation and Acute Lung Injury

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The relationship between local inflammation and the subsequent systemic inflammatory response is poorly described. In a burn injury model, the dermal inflammatory response may act as an ongoing trigger for the systemic inflammatory response syndrome (SIRS) and subsequent systemic complications. We hypothesized that topical attenuation of burn wound inflammatory signaling will control the dermal inflammatory source, attenuate SIRS, and reduce acute lung injury. Mice received a 30% total body surface area burn. Subgroups were treated with specific p38 MAPK inhibitor or vehicle, which was topically applied to wounds. Topical p38 MAPK inhibition significantly reduced burn wound inflammatory signaling and subsequent systemic expression of proinflammatory cytokines and chemokines. In vitro macrophage functional assays demonstrated a significant attenuation in serum inflammatory mediators from animals receiving the topical inhibitor. Topical p38 MAPK inhibition resulted in significantly less pulmonary inflammatory response via reduction of pulmonary neutrophil sequestration, pulmonary cytokine expression, and a significant reduction in pulmonary microvascular injury and edema formation. Although dermal activating transcription factor-2, a downstream p38 MAPK target, was significantly reduced, there was no reduction in pulmonary activating transcription factor-2 expression, arguing against significant systemic absorption of the topical inhibitor. These experiments demonstrate a strong interaction between dermal inflammation and systemic inflammatory response. Attenuating local inflammatory signaling appears effective in reducing SIRS and subsequent systemic complications after burn injury. *The Journal of Immunology, 2006, 177: 8065–8071.

The innate immune response is designed to focus a controlled inflammatory effort in a spatially localized manner. Subsequent systemic inflammation can be seen as an uncontrolled or overwhelming response. However, the relationship between local inflammation and subsequent systemic inflammatory response is unclear. This study investigates the effect of controlling local inflammatory signaling on systemic inflammatory response and subsequent complications such as pulmonary failure.

Burn injury robustly induces the dermal production of proinflammatory mediators, resulting in ongoing wound inflammation and tissue edema (1, 2). In addition to local inflammation, severe dermal burns are known to induce the systemic inflammatory response syndrome (SIRS)³, which correlates with a high risk of end organ failure as seen in burn-induced acute lung injury (ALI) (3, 4). In the absence of inhalational injury, burn wounds are the inflammatory source triggering SIRS. Several pathophysiological mechanisms are responsible for ALI and remote organ damage after local injury, such as the systemic liberation of proinflammatory mediators, neutrophil attraction, and activation of polymorphonuclear cell (PMN) trafficking, as well as potential activation of sympathetic inflammatory signaling (5–9). ALI and the adult respiratory distress syndrome top the list of early burn-induced complications and are associated with high mortality (4, 10). Therefore, therapies preventing the development of SIRS and subsequent ALI may significantly impact patient outcomes after burn injury.

p38 MAPK plays a prominent role in the regulation of inflammatory cell responses to extracellular stress and is elemental to proinflammatory signaling (11–14). We have shown previously that burn-induced dermal proinflammatory mediator production, dermal neutrophil sequestration, and microvascular injury were significantly attenuated after topical wound application of SB202190, an αβ-specific p38 MAPK inhibitor (15). Given the prominent role of burn wound inflammation in the systemic liberation of inflammatory mediators and activation of PMN trafficking, the current study was undertaken to determine whether early dermal inflammatory source control with topical agents would limit the extent of burn-induced SIRS and subsequent ALI.

We demonstrate that topical inhibition p38 MAPK in burn wounds significantly attenuates the dermal expression of the downstream p38 MAPK target activating transcription factor-2 (ATF-2) as well as the burn wound release of proinflammatory mediators (16). In contrast, no reduction in burn-induced pulmonary ATF-2 expression was seen, suggesting that no relevant systemic absorption of inhibitor occurred. The experimental intervention significantly attenuated the pulmonary inflammatory response.
Materials and Methods

Reagents

Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich.

Experimental animals

All experiments were performed in accordance with the guidelines set forth by the National Institutes of Health for care and use of animals. Approval for the experimental protocol was obtained from the University Committee on Use and Care of Animals at the University of Michigan. Age- and weight-matched female C57BL/6 black mice (The Jackson Laboratory) were individually housed. Sham animals (SH) were subjected to identical procedures or directly processed according to protocol.

Burn injury

C57BL/6 black mice were anesthetized via i.p. injection of 40 mg/kg pentobarbital (Abbott Laboratories). The dorsal hair was clipped, and animals were placed in an insulating mold device with an opening calculated to expose ~30% total body surface area (17). After immersion in 60°C water for 25 s, the burn area was scrub debrided with sterile gauze and rinsed with 0.9% sterile saline. Animals were reanesthetized with 4 ml/percentage of total body surface area/kg Ringer’s lactate i.p., received 0.3 mg/kg buprenorphine (Buprenex; Reckitt & Coleman Pharmaceuticals) s.c., and were individually housed. Sham animals (SH) were subjected to identical procedure and resuscitation, but immersed in room temperature water.

For topical inhibition of p38 MAPK, 0.5 ml of 10⁻⁴ M SB202190 (Calbiochem) every 8 h was applied to the wound, starting immediately after injury. The topical drug dose was determined in previous dose-response studies (15). SH and burn controls (BR; burn animal) received the drug vehicle consisting of a 4:1 mixture of acetone/olive oil, a combination widely used in topical applications (18). Wounds were covered with an occlusive dressing (Tegaderm; 3M Health Care). For organ harvest, animals were euthanized, and tissues were snap frozen in liquid nitrogen and stored at −80°C until further processing or directly processed according to protocol.

ELISA

For measurement of mouse IL-6, IL-1β, and MIP-2 by sandwich ELISA, we used Abs and standards from R&D Systems and 96-well micro plates (Immunoplate Maxisorb; Nunc) according to manufacturer’s instructions. A total of 100 µg of tissue was homogenized in 1000 µl of ice-cold lysis buffer consisting of 50 ml of 1× PBS with protease inhibitor (Complete X; Roche) and 50 µl of Triton X-100. Homogenates were centrifuged at 3000 × g for 5 min at 4°C; supernatants were stored at −80°C until use. Microplates were read using a microplate reader (BioTek Instruments) at 450 and 540 nm. Cytokine concentrations were calculated using a four-point standard curve; concentrations were adjusted for previous dilution and expressed as picograms per milliliter.

Microimmunoassay cytokine analysis

Serum proinflammatory cytokine levels were measured in tail vein blood via microimmunoassay analysis. For simultaneous detection of various cytokines, a total of 20 µl of blood was collected in 80 µl of EDTA. The system was developed in our laboratory and simultaneously measures up to 21 different cytokines. A total of 333–350 pl of each monoclonal capture Ab (500–1000 µg/ml) was spotted on 16 pad nitrocellulose membrane slides (Grace Biolabs) using Piezorray (PerkinElmer) (19). Slides were blocked with Protein Array Blocking Buffer (Schleicher & Schuell Microscience) for 1 h, and then incubated with 0.090 ml of cytokine standards (samples) for 2 h. After interval washing with wash buffer, biotin-conjugated anti-cytokine Abs were added to slides and incubated for 2 h. Following extensive washing, Cy5-conjugated streptavidin was added to slides and incubated for 30 min. After washing, slides were dried and scanned using Axon Genepix 4000. A cytokine standard curve was generated using STATLIA Program (Brendon Scientific).

Isolation and detection of lung IL-6, TNF-α, and inducible NO synthase (iNOS) mRNA by quantitative real-time PCR

A total of 100 mg of fresh tissue was homogenized in 1 ml of TRIzol (Invitrogen Life Technologies) and processed using the guanidine isothiocyanate/chloroform extraction method according to the manufacturer’s
guidelines. This was followed by spectrophotometric RNA quantification. For mRNA target gene amplification, a thermal cycler (Smart Cycler; Cepheid) was programmed, as follows: a hot start at 95°C for 2 min, followed by 43 amplification cycles with 95°C melting temperature (15 s), 60°C annealing temperature (15 s), and extension at 72°C (30 s). The following primers were used: IL-6, forward: 5′-ACTTCACAGAGGATACCACTC-3′, reverse: 5′-CTCCCTTTGCGAAGACTCA GG-3′; iNOS forward: 5′-AAGTCTCAGACATGGCTTGCC-3′, reverse: 5′-TGATCCTCACATACTGTGGAC-3′; β-actin was used as housekeeping gene: forward, 5′-TTCTACCAGATGGCTGGCC-3′; reverse, 5′-AAG AAGGAAGCTGAAAG-3′. All primers were purchased from Invitrogen Life Technologies. SYBRGreen threshold (cycle threshold; CT) was used (Polytron; KINEMATICA). Used lysis buffer contained 20 mM Tris HCl, 0.1% sodium deoxycholate, 0.5 M EDTA, and 1% Triton X-100 (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, and 1 μg/ml leupeptin; dermal and pulmonary phosphorylated ATF-2 was measured using a prefabricated sandwich ELISA kit (PathScan Phospho-ATF-2; Cell Signaling Technology) that detects endogenous levels of phospho-ATF-2 (Thr381) protein via spectrophotometric detection at 450 nm.

For assessment of microvascular injury, animals were anesthetized 30 min before the scheduled harvested time point; 50 mg/kg body weight of EB was injected via tail vein. At times 6 and 12 h postinjury, animals were exsanguinated by incision of the right atrium. Systemic circulating dye was eluted, inserting a 20G cannula through the apex of the left ventricle into the ascending aorta and perfusing a total volume of four times the calculated blood volume (7.46 ml/100 g), as described previously (20), using heparinized (100 U/ml) 0.9% saline and a pressure-controlled perfusor pump (B. Braun Medical) at constant flow rate. Pulmonary intravascular dye was eluted via pulmonary artery cannulation and perfusion with 5 ml of heparinized saline. Lungs were harvested, weighed, and placed in 4 ml of 99.5% formamide/g tissue. After 48 h at room temperature, supernatants were collected and spectrophotometrically analyzed, comparing the measured values to an EB standard curve and formamide blanks. The results are expressed as μg of EB/mg tissue.

**Quantification of microvascular injury (Evans blue; EB)**

**Determination of pulmonary edema (wet/dry weight)**

Fresh lung tissue was weighed; 100-mg samples were placed in an incubator and dried at 60°C for 48 h. The dry weight of each sample was measured, and the wet-dry weight ratio was calculated from the mean weights of all animals per group.

**Measurement of phosphorylated ATF-2**

For quantification of skin and lung tissue, an electric homogenizer was used (Polytron; KINEMATICA). Used lysis buffer contained 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, and 1 μg/ml leupeptin; dermal and pulmonary phosphorylated ATF-2 was measured using a prefabricated sandwich ELISA kit (PathScan Phospho-ATF-2; Cell Signaling Technology) that detects endogenous levels of phospho-ATF-2 (Thr381) protein via spectrophotometric detection at 450 nm.

**In vitro macrophage stimulation assay**

RAW 264.7 cells, a mouse peritoneal macrophage cell line, were obtained from the ATCC. The cells were maintained in RPMI 1640 with 10% FBS, 100 U/ml penicillin/streptomycin, and 2 mM L-glutamine. Cells were grown to 60/70% confluence, counted via Coulter counter (Coulter Electronics), and cultured in 6-well tissue culture plates. Once cells numbered between 3.5 × 10^6 and 5.0 × 10^6/well, they were washed with PBS, and placed in serum-free RPMI 1640. Duplicate wells were precultured with 5% serum from vehicle-treated SH or BR or 5% serum from topical SH or BR treated animals before being stimulated with either 10 or 100 ng of LPS (Escherichia coli 0111:B4). Supernatants were collected and stored on ice for immediate assay performance. Samples were run in triplicate with 20 μl of sample and 200 μl of substrate buffer (containing in addition to 1 M monobasic and dibasic potassium phosphate 0.3% hydrogen peroxide and o-dianisidine HCl) per well. A plate reader was programmed to measure kinetic ΔOD changes in 10-s intervals at 456 nm for 90 s (Synergy HT; BioTek Instruments). Data are expressed as ΔOD/mg tissue/min.

**FIGURE 3.** Role of soluble inflammatory mediators in burn-induced systemic inflammation. RAW cells were preincubated in 5% serum from SH (SH), BR receiving topical vehicle (BR), and animals receiving topical SB202190 (BR + SB) for 30 min before LPS stimulation with either 10 or 100 ng. TNF-α expression by RAW cells in BR + SB was significantly less than BR group after 10 ng of LPS stimulation. (Mean ± SEM; p < 0.05; BR vs BR + SB; ANOVA.) Experiments were done in duplicates with a minimum of n = 5 animals/group.

**FIGURE 4.** Inflammatory source control in dermal burn wounds reduces pulmonary IL-6, TNF-α, and iNOS target gene expression. Pulmonary mRNA was isolated from SH (SH + Veh and SH + SB), burn with vehicle treatment only (BR + Veh), and burn wounds treated with topical p38 MAPK inhibitor SB202190 (BR + SB) 48 h after injury. IL-6 (A), TNF-α (B), and iNOS (C) target gene levels were assessed using quantitative RT-PCR. Burn injury induced pulmonary expression of all three target genes. Significant reduction of IL-6 and iNOS target gene mRNA after topical p38 MAPK inhibition was seen, and TNF-α showed a marked reduction in pulmonary expression. Experiments were repeated three times (n = 6/group/time point; mean ± SEM; *, p < 0.05, ANOVA).
collected after 1 h, and TNF-α expression was measured by ELISA (R&D Systems).

**Statistical analysis**

All values are expressed as mean ± SEM. Data were analyzed using Student’s t test and one way ANOVA, followed by Bonferroni post hoc testing (GraphPad). Statistical significance was set at a p value <0.05.

**Results**

**Attenuation of dermal inflammatory response in burn wounds using topical treatment**

As early as 1 h after burn injury, a significant local inflammatory response was found with elevations in dermal IL-6 and IL-1β in BR. Application of topical p38 MAPK inhibitor attenuated dermal inflammation. There was a significant rise in dermal IL-6 and IL-1β in burned mice (BR) receiving topical vehicle compared with sham group animals (SH) receiving vehicle. In contrast, mice subjected to burn injury and given the topical inhibitor (burn and topical SB202190 application (BR plus SB)) showed a significant inhibition of dermal IL-6 at 4 and 12 h postburn (BR plus Veh vs BR plus SB; 4 and 12 h; p < 0.01) (Fig. 1A). Similarly, IL-1β levels were significantly inhibited at 1 and 4 h postburn (1 h BR plus Veh vs BR plus SB, p < 0.01; 4 h, p < 0.01) (Fig. 1B). Treating SH with either topical vehicle or vehicle with SB202190 resulted in no difference in dermal proinflammatory cytokine production.

**Circulating proinflammatory cytokines and inflammatory mediators are reduced after topical modulation of burn wound inflammation**

Twenty-four hours after burn injury, serum proinflammatory cytokine analysis demonstrated a significant increase in IL-6 and IL-1β levels in serum of BR vs SH. At 24 h postburn, IL-6 levels were significantly reduced in the treatment group (BR plus Veh vs BR plus SB; p < 0.05) (Fig. 2A). Compared with BR, the treatment group had reduced levels of IL-1β (Fig. 2B). There was no significant difference between IL-6 and IL-1β levels in SH treated with either vehicle of topical inhibitor.

To assess the overall immunomodulatory effect of the serum, RAW 264.7 cells were preincubated in serum obtained from animals 6 h after sham or thermal injury. RAW cells preincubated in serum from BR had a significantly higher TNF-α production compared with sham serum when stimulated with either 10 or 100 ng of LPS (SH vs BR; p < 0.001). However, RAW cell preincubation with serum of BR treated with topical p38 inhibitor had a significantly lower TNF-α expression after LPS stimulation compared with vehicle-treated group (Fig. 3).

**FIGURE 5.** Attenuation of burn wound inflammation reduces pulmonary neutrophil sequestration and MIP-2 production. Lungs from SH (SH + Veh and SH + SB), burn with vehicle treatment only (BR + Veh), and burn wounds treated with topical p38 MAPK inhibitor SB202190 (BR + SB) were homogenized 1, 4, 12, and 24 h postburn. MPO assay (A) and MIP-2 ELISA (B) performed on tissue homogenate demonstrated peaked activity 12 h postinjury. Topical SB202190 application significantly reduced MPO and MIP-2 levels at times 12 and 24 h postinjury. Experiments were repeated three times with a minimum of n = 5/group/time point. (Mean ± SEM; *, p < 0.01; BR + Veh vs BR + SB; ANOVA.)

**FIGURE 6.** Reduction of inflammatory signaling in burn wounds reduces pulmonary microvascular injury and edema. Thirty minutes before harvest SH (SH), burn with vehicle treatment only (BR), and burn wounds treated with topical p38 MAPK inhibitor SB202190 (BR + SB) received systemic 50 mg/kg EB. Tissue-bound EB was extracted from lungs (A) with formamide and measured by spectrophotometry (B). Burn injury induced significant pulmonary albumin leakage at 6 and 12 h postburn (BR plus Veh vs BR plus SB, 4 and 12 h; p < 0.01) (Fig. 1A). Similarly, IL-1β levels were significantly inhibited at 1 and 4 h postburn (1 h BR plus Veh vs BR plus SB, p < 0.01; 4 h, p < 0.01) (Fig. 1B). Treating SH with either topical vehicle or vehicle with SB202190 resulted in no difference in dermal proinflammatory cytokine production.

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Dermal inflammatory source control reduces pulmonary proinflammatory cytokine and iNOS target gene expression

IL-6, TNF-α, as well as iNOS are known key players in ALI. The pulmonary expression of all three target genes was significantly enhanced after burn injury (Fig. 4). In contrast, modulating the inflammatory source in the burn wound with the topical agent significantly reduced IL-6 (BR plus Veh vs BR plus SB; p < 0.05) and iNOS target gene expression (BR plus Veh vs BR plus SB; p < 0.05). Additionally, a reduction of TNF-α target gene expression was noted, which did not reach statistical significance (p = 0.09).

Topical inhibition of p38 MAPK in burn wounds attenuates pulmonary neutrophil sequestration and pulmonary MIP-2 levels

We assessed pulmonary neutrophil sequestration as an early marker of pulmonary inflammation and examined the expression of MIP-2, a strong neutrophil chemoattractant produced mainly by alveolar macrophages. There was a sustained rise in pulmonary MPO activity, starting at 4 h postinjury with a peak at 12 h; MPO activity remained elevated up to 24 h postburn. A significant attenuation of neutrophil sequestration was seen at 12 and 24 h (both time points: BR plus Veh vs BR plus SB; p < 0.01) after injury (Fig. 5A).

There was a parallel rise in pulmonary MIP-2 after burn injury in BR. The treatment group demonstrated significantly inhibition at 12 and 24 h (both time points: BR vs BR plus SB; p < 0.01) postburn (Fig. 5B).

Modulating p38 MAPK signaling in burn wounds reduces pulmonary microvascular injury and reduces pulmonary edema

Microvascular damage was quantified by measuring tissue extravasation of albumin bound EB. Early postinjury, there was substantial EB extravasation (Fig. 6A), which was significantly attenuated with topical p38 MAPK inhibitor at 6 h (BR vs BR plus SB; p < 0.05) and 12 h (BR vs BR plus SB; p < 0.01) postburn (Fig. 6B).

As expected, pulmonary edema formation followed this increase in microvascular leakage. Measuring pulmonary wet and dry weights allows experimental assessment of pulmonary edema. Lungs harvested at 6 h postburn showed no difference in wet: dry ratios (Fig. 6C). However, at 12 h postinjury, we noted a significant rise of wet: dry ratios in burn lungs (BR). Pulmonary edema was significantly reduced in animals receiving BR plus SB (BR vs BR plus SB; p < 0.05).

Topical p38 MAPK inhibition attenuates burn-induced dermal, but not pulmonary ATF-2 expression

ATF-2 is a well-known downstream target for activated p38 MAPK (16). Because SB202190 efficiently inhibits p38 MAPK function, presence of this compound should result in a significant decrease in tissue ATF-2 levels. As expected, topical inhibitor application (BR plus SB) significantly reduced dermal ATF-2 expression at 1 h postinjury compared with vehicle (BR plus Veh) (p < 0.05) (Fig. 7A). However, topical SB202190 did not inhibit pulmonary ATF-2 expression (Fig. 7B). At 8 h postinjury, all the BR, including the topical vehicle- and SB202190-treated groups, showed significant activation of pulmonary ATF-2 compared with SH (p < 0.05). The pulmonary ATF-2 expression was not inhibited by application of topical p38 MAPK inhibitor, indicating that there was no significant systemic absorption.

Discussion

Skin, a highly immunocompetent organ, reacts to burn injury via a complex inflammatory response (21, 22). Although dermal inflammation is an important physiologic part of wound healing, excessive local wound inflammation may activate systemic inflammation. Severe burn injuries are known for ongoing and uncontrolled hyperactivation of various host defense mechanisms resulting in SIRS, multiorgan failure, and high patient mortality (23, 24). The local dermal inflammatory process acts as a lasting trigger, stimulating SIRS via production of cytokines and other inflammatory mediators, the activation of neutrophil trafficking, and potentially neural proinflammatory stress signaling (5, 7, 9, 25). In this scenario, dampening excessive inflammation can be a warranted strategy to limit injurious host response to a noninfectious process. In our approach, we targeted the local dermal inflammatory process that is driving SIRS. We hypothesized that by limiting the source of inflammation we can modify the systemic response and reduce end organ failure.

We previously demonstrated the feasibility of attenuating dermal inflammation after thermal injury by topical application of SB202190, an α-/β-p38 MAPK inhibitor in rats (15). In the current set of experiments, we start out by demonstrating that modulating p38 MAPK expression in burn wounds also reduced dermal inflammation in a mouse model. As expected, burn injury induced a subsequent systemic inflammation with activation of inflammatory response in distant organs, such as lungs. Our data revealed a lasting up-regulation of proinflammatory cytokines in serum after thermal injury. Topical inhibition of burn wound inflammatory signaling significantly reduced serum levels of IL-6 and attenuated IL-1β levels. In rodents, a burn-induced elevation of proinflammatory cytokines in serum and its correlation with ALI are well
described (26, 27). Supportive data exist from clinical studies regarding the predictive value of elevated serum levels of proinflammatory cytokines for development of ALI/acute respiratory distress syndrome and associated patient mortality (28, 29). The role of soluble inflammatory modulators in our model is supported by an in vitro functional cell assay. RAW cells preincubated in 5% serum from BR receiving topical SB202190 had significantly lower TNF-α expression compared with topical vehicle-treated group. These data overall demonstrate a significant reduction in systemic inflammatory mediators in the burn wound and serum of animals treated with topical p38 MAPK inhibitor.

The onset of pulmonary production of various inflammatory mediators is seen by many authors as an indicator of established lung inflammation (30). The injured lung becomes a potent inflammatory source in itself, accelerating the development of SIRS (31). Pulmonary cytokine and chemokine expression as well as PMN sequestration were significantly reduced in BR treated with topical p38 MAPK inhibitor. A reduction in ATF-2, a downstream target for p38 MAPK, confirmed the effectiveness and extent of topical p38 MAPK inhibition. Presence of SB202190 in the tissue is expected to significantly inhibit ATF-2 expression. As expected, there was a significant dermal inhibition of ATF-2 activation at 1 h postburn. At 8-h time point, the inhibition by topical p38 MAPK treatment persisted, albeit the effect seemed less prominent, possibly due to the t1/2 of the drug. In contrast, at both time points, topical inhibition had no effect on pulmonary ATF-2 activation, supporting the importance of a local-dermal effect of p38 MAPK inhibition. More so, this finding suggests that there is no significant systemic absorption of the topical inhibitor, which would be otherwise a potential explanatory mechanism for the attenuating effect on ALI. Topical MAPK inhibitor application has to date only been described in dermatological literature as a feasible strategy to deliver antiallergic drugs locally to reduce development of contact allergies in mice (18). To our knowledge, topical inhibition of p38 MAPK as a strategy for inhibition of end organ injury in burns has not been investigated yet. Moreover, the study methodology can be incorporated into current clinical practice. On admission, burn patients undergo debridement of the necrotic epidermis and application of a topical antimicrobial agent with subsequent repeated application once or twice daily. Therefore, early application of the topical p38 inhibitor with the topical antimicrobial agent is clinically plausible.

The presented data support the role of burn wound inflammation as the source for ongoing systemic stimulation and development of SIRS and the protective effect of topical inflammatory source control. Our favorable results after topical p38 MAPK inhibition in dermal burns are in support of a source-specific mode of drug application. Growing evidence shows that indiscriminate systemic p38 MAPK inhibition may have unpredictable consequences (32, 33). Although some studies demonstrated decreased pulmonary inflammation in endotoxin-induced ALI (34, 35), others have reported increased pulmonary inflammation in response to endotoxemia or hemorrhage and do not endorse systemic p38 MAPK inhibition as a therapeutic strategy (36). It is interesting in this context to note that systemic p38 MAPK inhibition was found to attenuate LPS-induced TNF-α production in whole blood, but the same model noted increased peritoneal macrophage inflammatory response, a finding that further underlines the tissue specificity of p38 MAPK activation (37).

In summary, topical p38 MAPK inhibition in burn wound appears to be an effective strategy to dampen systemic inflammation by controlling the source of peripheral inflammatory signaling. Our results demonstrate the importance of burn-induced dermal inflammation in the development of ALI via soluble inflammatory mediators. Reducing acute wound inflammatory signaling could potentially improve patient outcomes via reduction of early inflammatory end organ injury. This novel concept could be readily transferred to clinical settings due to its proximity to current practice of topical application of antimicrobials in burns.

Disclosures
The authors have no financial conflict of interest.

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