Burn Injury–Induced Alterations in Wound Inflammation and Healing Are Associated with Suppressed Hypoxia Inducible Factor-1α Expression

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A major complication associated with burn injury is delayed wound healing. While healing of the burn injury site is essential, healing of distal injury sites caused by surgical interventions and other processes also is important. The impact of burn injury on healing of these distal wound sites is not understood clearly. To study this, mice were subjected to major burn injury or a sham procedure. Immediately following, excisional wounds were made on the dorsal surface caudal to the burn site and wound closure was monitored over a 7-d period by planimetry. In a second series of experiments, plasma and excisional wounds were collected for *in vitro* analysis of cyto- and chemokine levels, L-arginine metabolism, and hypoxia-inducible factor (HIF)-1α expression. At 1–7 d post-injury, a significant inflammatory response was evident in both groups, but the healing process was delayed in the burn-injured mice. At 3 d post-injury, wound levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and keratinocyte-derived chemokine were suppressed in the burn group. This difference in the wound inflammatory response was independent of changes in L-arginine metabolism (nitrate levels, inducible nitric oxide synthase expression, arginase activity), but correlated with a marked reduction in HIF-1α protein levels. In conclusion, these findings suggest that HIF-1α and the inflammatory response play a significant role in wound healing, and reduced levels of HIF-1α contribute to the impaired healing response post-burn.

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INTRODUCTION

Complications induced by burn injury include immunosuppression, sepsis, and delayed wound healing. Even with the development of improved burn patient care, many problems which develop for burn patients are associated with the healing process. Wound healing is a complex process involving a series of overlapping phases, including inflammation (1–3). Wound healing is an intricately regulated sequence of cellular and biochemical events orchestrated to restore tissue integrity after injury. Immune/inflammatory cells have an integral function in wound healing beyond their role in inflammation and host defense, as they are essential to the regulation of the wound-healing process through the secretion of signaling molecules, such as cytokines, chemokines, and growth factors (4–6). Dysregulation and dysfunctional activation of the immune/inflammatory systems is a significant hallmark of the response to burn injury (7,8). While the role of inflammatory cells in wound healing is well established, evidence for both positive and negative effects has been suggested. In this regard, recent investigations support the paradigm that robust inflammation, such as that associated with burn injury, may be detrimental to wound closure (9).

Early burn wound excision and grafting is common clinical practice, and studies have shown that it can result in decreased morbidity and mortality (10–13). Skin grafting, however, by necessity, creates a secondary wound site, (that is, the graft donor site). Complications at the healing graft donor site, pain/discomfort, infection, and slow healing can contribute to patient morbidity. Characterization of the inflammatory response at the graft donor site, a secondary wound site, has not been investigated either experimentally or clinically. Elucidation of the processes involved in the healing of the burn graft donor site are of significant value in developing improved therapeutic regimes for burn patients. The current study was undertaken to investigate the impact of burn injury

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on the healing and inflammatory response in a secondary dermal injury site, similar to that of a skin graft donor site.

MATERIALS AND METHODS

Animals

C57BL/6 male mice, 18–22 g, 8–10 wks of age (Charles River Laboratories, Wilmington, MA, USA) were used for all experiments. The mice were allowed to acclimatize in the animal facility for at least 1 wk prior to experimentation. Animals were assigned randomly to either a dual injury group of thermal injury with excisional dermal wounding (Burn/EW) or an excisional wound only group (EW). The experiments in this study were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham, Birmingham, AL, USA (where the experiments were conducted), and were performed in accordance with the National Institutes of Health guidelines for the care and handling of laboratory animals.

Thermal Injury Procedure

Mice received a scald burn as described previously (14). Briefly, the mice were anesthetized by intra-peritoneal injection of ketamine/xylazine and the dorsal surface was shaved. The anesthetized mouse was placed in a custom insulated mold exposing 12.5% of its total body surface area (TBSA) along the right dorsum. The mold was immersed in 70° C water for 10 s, producing a burn injury. The mouse was repositioned in the mold, with the left dorsum exposed; and the mold was reimmersed in 70° C water for 10 s, with the result being a major burn injury covering 25% of the TBSA. The mice were resuscitated with 1 mL of Ringer's lactate solution administered by intra-peritoneal injection and returned to their cages. The cages were placed on a heating pad for 2 h until the mice were fully awake, at which time they were returned to the animal facility. Sham treatment only consisted of anesthesia and resuscitation with Ringer's lactate solution.

Excisional Wound Procedure

Excisional wounds (EW) on the caudal dorsal surface were induced as described by DiPietro and coworkers (15). In brief, following the induction of thermal injury or sham procedure, two full-thickness excisional dermal wounds were made using an 8 mm biopsy punch (Miltex, York, PA, USA). Care was taken to avoid the dorsal vessels during injury by elevating the redundant skin and using trans-illumination to identify the vessels. Hemostasis was obtained by applying pressure to the wound for 1–2 min.

Measurement of Wound Closure

Direct measurements of wound area and wound closure were determined from tracing of the wounds onto a transparent plastic sheet at 0–7 d post-injury. The wound tracings were digitalized and analyzed using planimetry software (ImageJ, NIH). Data are presented as percentage (%) change from original injury size.

Collection and Processing of Plasma and Excisional Wound Samples

At 1–7 d after injury, blood was collected by cardiac puncture, and plasma separated and stored at –80° C until analysis. Wounds were excised down to the level of the musculofascia by sharp dissection and included the surrounding wound margin tissue (16). The samples were snap-frozen immediately in liquid nitrogen and stored at –80° C until analysis. Tissue samples were homogenized in protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) prior to analysis as described elsewhere (17).

Analysis of Cytokine and Nitrite Levels

The concentrations of interleukin (IL)-6, IL-10, monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF)-α in the wound homogenates and plasma were measured by commercially available cytometric bead array (CBA) Mouse Inflammation Kits (BD Pharmingen, San Diego, CA, USA), according to the manufacturer's instructions, with some modification. Briefly, 25 μL of mixed capture beads were incubated with 25 μL of supernatant and 25 μL of Phycoerythrin (PE) detection reagent for 2 h at room temperature. Then, the immunocomplexes were washed and analyzed using the LSRII flow cytometer (BD Biosciences, Mountain View, CA, USA). The data was processed with the accompanying FACSDiva and BD CBA software. Similarly, keratinocyte-derived chemokine (KC) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were measured by CBA Mouse Flex Sets (BD Pharmingen) according to the manufacturer's instructions with some modification. In brief, 25 μL of mixed capture beads were incubated with 25 μL of skin homogenates, wound fluid or culture supernatant for 1 h at room temperature in the dark. Twenty-five μL of PE detection reagent was then added and incubated for 1 h. Then, the immunocomplexes were washed twice with wash buffer and centrifuged at 200*g* for 5 min. Analysis was carried out using the LSRII flow cytometer (BD Biosciences) and the data was processed with the accompanying FACSDiva and FCAPArray software. Cytokine concentrations in the skin homogenates were normalized against protein content. Tissue growth factor (TGF)-β1 levels were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) according to the manufacturer's recommendations (R & D Systems, Minneapolis, MN, USA). Samples were acidified prior to analysis to activate latent TGF-β. Nitrite/nitrate levels were determined using the Greiss reaction using a kit from Cayman Chemical (Ann Arbor, MI, USA). Values for wound samples were normalized to μg of total protein as determined using a BioRad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA).

Hypoxia Inducible Factor (HIF)-1α Analysis

HIF-1α levels in wound homogenates were determined by ELISA according to the manufacturer's recommendations (R & D Systems) and by Western blot as

described below. Values were normalized to μg of total protein.

Western Blot Analysis for HIF-1α and Inducible Nitric Oxide Synthase (iNOS)

Approximately 100 mg of frozen tissue from each animal were homogenized in 1 mL of lysis buffer (50 mM HEPES, 10 mM sodium pyrophosphate, 1.5 mM $MgCl_2$, 1 mM EDTA, 0.2 mM sodium orthovanadate, 0.15 M NaCl, 0.1 M NaF, 10% glycerol, 0.5% Triton X-100 and protease inhibitor cocktail). The lysate supernatants (50 μg per lane) were then mixed with 4 × sodium dodecylsulfate (SDS) sample buffer. Samples were electrophoresed on 4%–12% SDS-polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) and transferred onto nitrocellulose membranes (Invitrogen) at 35 *V* for 60 min. The membranes were blocked with 5% nonfat dry milk and then immunoblotted with the following primary antibodies: iNOS (1:1000; Cell Signaling Technology, Beverly, MA, USA); HIF-1α (1:1000; Abcam, Cambridge, MA, USA) or GAPDH (1:25,000; Abcam) overnight at 4° C. After washing with TBST, the membranes were incubated with HRP-conjugated goat anti-rabbit or anti-mouse IgG secondary antibody for 1 h at room temperature and developed by enhanced chemiluminescence (Amersham, Piscataway, NJ, USA). Quantification was performed using ImageJ imaging software (NIH).

Arginase Activity Assay

Arginase activity in wound homogenates was determined using a QuantiChrom arginase assay kit according to the manufacturer's instructions (BioAssay Systems, Hayward, CA, USA). Values were normalized to μg of total protein.

Statistical Analysis

Data are expressed as mean ± SEM. Comparisons were analyzed using analysis of variance (ANOVA) and Tukey's test for multiple comparisons. A *P* value of < 0.05 was considered to be statistically significant for all analyses.

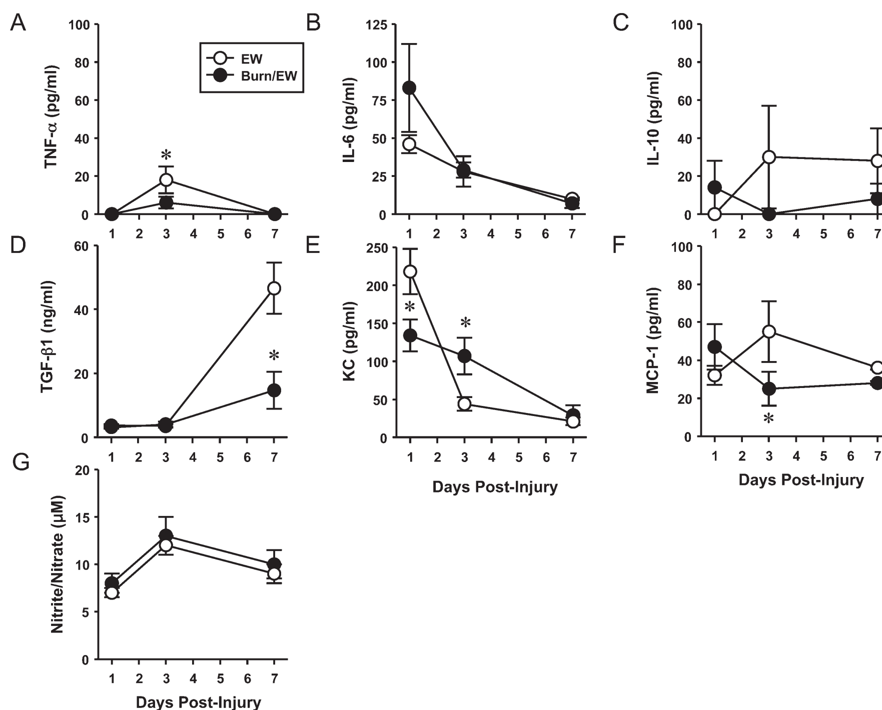

Figure 1. Plasma cytokine, chemokines, and nitrite/nitrate levels. Mice were subjected to an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW) and plasma samples collected at 1–7 d post-injury. Plasma levels of cytokines (A–D), chemokines (E,F), and nitrite/nitrate (G) were determined as described in the Materials and Methods section. Data are mean ± SEM; *n* = 3–7 mice/group. **P* < 0.05 as compared with EW group.

RESULTS

Effect of Injury on the Systemic Inflammatory Response

Plasma samples were collected from mice at 1, 3, and 7 d post-injury to determine the impact of excisional wounding (EW) independent of burn injury on the systemic inflammatory response. The results shown in Figure 1 indicate that EW, with or without burn injury, induced a significant inflammatory response. Significant levels of IL-6, KC, MCP-1, and nitrite/nitrate were evident in the plasma of both groups at 1 d post-injury. A trend toward increased IL-6 was observed in the burn/EW group; however, statistical significance was not evident due to the limited number of samples analyzed. In both groups, IL-6 and KC levels declined at 3–7 d post-injury. In contrast, MCP-1 levels remained relatively unchanged and nitrite/nitrate levels increased at 3–7 d post-injury. A slight elevation in TNF-α levels was evident at 3 d post-injury whereas TGF-β levels increased at 7 d post-injury in both groups. Plasma IL-10 levels were not elevated by injury in either group. With regard to the combined injury (Burn/EW), TNF-α and MCP-1 levels were significantly lower at 3 d post-injury, whereas KC levels were significantly elevated as compared with the excisional wounds alone. TGF-β plasma levels were significantly greater at 7 d post-injury in the EW group as compared with the Burn/EW group. IL-6, IL-10, and nitrite/nitrate levels were not different between groups at any time points.

Excisional Wound Healing

Closure of the excisional wounds was monitored over a 7-d period (Figure 2).

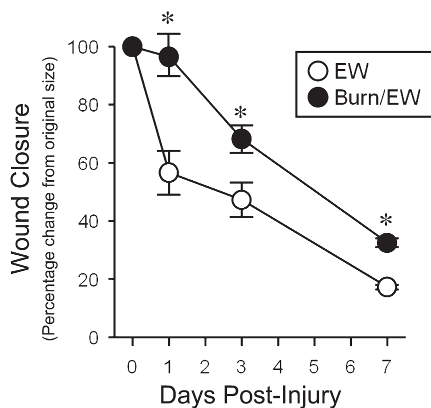

Figure 2. Time course of wound closure. Mice were subjected to an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW) and wound closure was determined 1–7 d post-injury as described in the Materials and Methods section. Data are mean ± SEM; *n* = four mice/group. **P* < 0.05 as compared with EW group.

In the EW group, significant closure was evident as early as 24 h after injury with approximately a 50% decrease in wound size. In contrast, significant closure of the wound in the Burn/EW group was not evident until 3 d post-injury. The degree of wound closure was significantly less in the Burn/EW group at all time points assessed. The images in Figure 3 show representative wounds at 3 d post-injury for both groups. Qualitatively, the EW group displays greater contracture than the Burn/EW group, as evidenced by the irregularity in the wound shape.

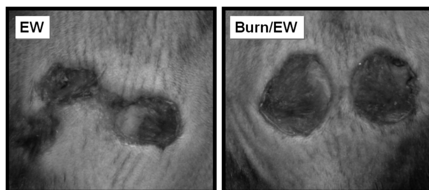

Figure 3. Wound closure at 3 d post-injury. Mice were subjected to an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW). Digital photographs were taken of representative animals at 3 d post-injury.

Excisional Wound Cytokine and Chemokines Content

The analysis of the wound cyto- and chemokines content was done at 3 d post-injury, as the initial analysis of the systemic inflammatory response showed the greatest differences between the groups at 3 d post-injury and, by d 3, both groups displayed significant wound healing. Wounds from both the EW and EW/Burn groups contained significant levels of all cyto- and chemokines evaluated with the exception of IL-10 (Figure 4). Wound homogenates from the Burn/EW group contained significantly lower levels of the cytokines TNF-α, IL-6, and the chemokine KC as compared with the EW group. The reduction in cyto-/chemokine content ranged from approximately 25% for KC to 50% for TNF-α. No differences in the wound content of MCP-1 or TGF-β were observed between the groups.

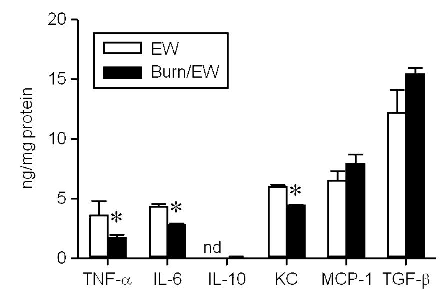

Figure 4. Excisional wound cytokine and chemokine levels. At 3 d post-injury, excisional wounds were collected from mice receiving an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW). Wound homogenates were generated and cytokine and chemokine levels were determined as described in the Materials and Methods section. Levels for TNF-α IL-6, IL-10, KC, MCP-1, and TGF-β were determined. Data are mean ± SEM; *n* = four mice/group. **P* < 0.05 as compared with EW group.

Wound L-Arginine Metabolism

Wound L-arginine metabolism was assessed by measuring nitrite/nitrate levels, iNOS protein expression, and arginase activity in the wound homogenates. As anticipated, at 3 d post-injury significant levels in nitrite/nitrate, iNOS expression, and arginase activity were evident in the wound homogenates of both the EW and the Burn/EW group (Figure 5). No difference in these parameters of L-arginine metabolism was observed between groups.

Wound HIF-1α Protein Expression

HIF-1α levels in the wound homogenates was determined by both ELISA and Western blot analysis (Figure 6). As determined by ELISA, a profound loss of HIF-α expression was observed in the Burn/EW group. HIF-1α expression was approximately 25-fold lower in the Burn/EW group as compared with the EW group. Western blot analysis revealed a similar, but less dramatic (~6-fold) loss of HIF-1α protein expression in the Burn/EW group.

DISCUSSION

In the current study a dual injury of burn and excisional wounds markedly attenuated the closure of the excisional wound sites. In parallel, the post-injury inflammatory response was suppressed in the animals receiving dual injuries. This was evidenced by lower systemic levels of: 1) KC at d 1 post-injury; 2) TNF-α and MCP-1 at d 3 post-injury, and; 3) TGF-β at d 7 post-injury. In addition, a suppressed inflammatory response at the excisional wound site was observed in the dual injury group with decreased tissue levels of TNF-α, IL-6, and KC. These changes in wound healing and wound inflammation were independent of various parameters of L-arginine metabolism (that is, nitrate, iNOS, and arginase), but were associated with a profound attenuation of HIF-1α expression in the dual injury group.

The wound healing process is complex, involving a series of overlapping phases including hemostasis, inflammation, proliferation, and resolution (1–3). The delayed wound healing observed in the dual injury group may be due in part to a lack of a sufficient inflammatory response at the excisional wound site. We have shown previously that a lack of an inflammatory infiltrate at the burn site

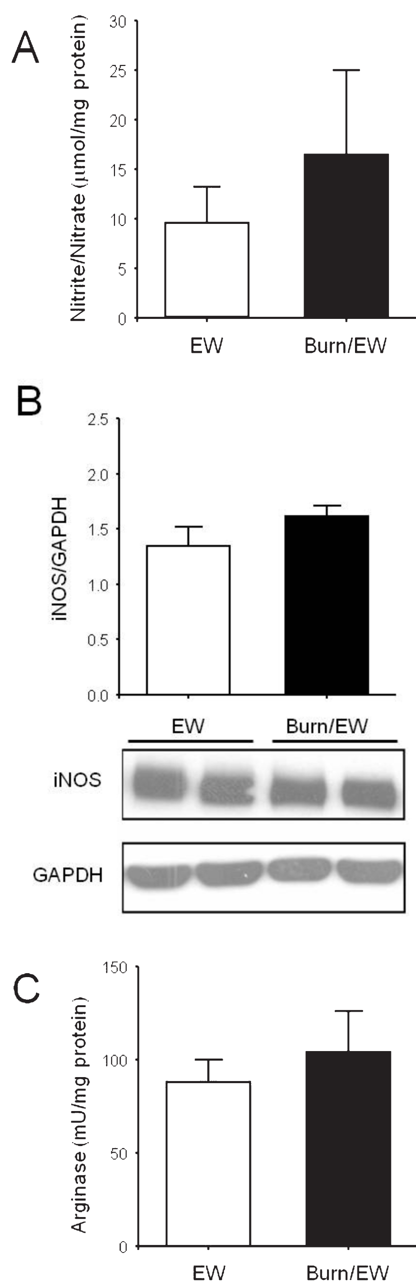

Figure 5. Excisional wound L-arginine metabolism. At 3 d post-injury, excisional wounds were collected from mice receiving an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW). Wound homogenates were assessed for various parameters of L-arginine metabolism: (A) nitrite/nitrate levels; (B) iNOS protein expression and; (C) arginase activity as described in the Materials and Methods section. Blots are of representative experiments. Data are mean ± SEM; *n* = four mice/group.

profoundly delays healing (17). While we did not assess cellular infiltration of the injury site specifically in the current study, the decreased levels of KC and MCP-1, (chemokines for neutrophils and monocytes, respectively) suggest that the movement of immune cells into the injury site might be retarded. A possible reason for this may be the active inflammatory response at the burn injury site overwhelming the ability of the animal's immune system to mount an effective response at the secondary excisional wound site. Our previous work has demonstrated that, in our model, there is a profound inflammatory response at the burn injury site at 3 d post-injury (18).

The two major pathways for L-arginine metabolism are the catabolic enzymes, nitric oxide synthase (NOS), and arginase, which metabolize L-arginine to produce nitric oxide (NO) and L-citrulline or urea and L-ornithine, respectively (19). The lack of any difference between the injury groups in this study indicates that the suppressed healing and inflammatory response in the dual injury group of burn/EW is independent of changes in NO generation and arginase activity.

The lack of oxygen (hypoxia) is an important signal in wound healing, as it regulates cellular proliferation, cellular migration, induction of cytokine production, and various intracellular signaling pathways (20). HIF-1 plays a critical role in the hypoxia-induced expression of these cytokines and growth factors (20,21). However, it has been suggested that dual pathways are involved in the induction of HIF-1 within the wound bed. Pro-inflammatory mediators stimulate early expression, whereas, wound hypoxia becomes the major stimulus at later time points (20,22,23). The induction of HIF-1α expression at the wound site in the current study is related to the early inflammatory response and, in the excisional wound, animals correlated with a strong inflammatory response. The dual injury animals with burn and excisional wounds with an attenuated inflammatory response had profoundly lower HIF-1α expression, independent of ELISA or Western blot analysis. However, since the decrease in HIF-1α was proportionally much greater than that of

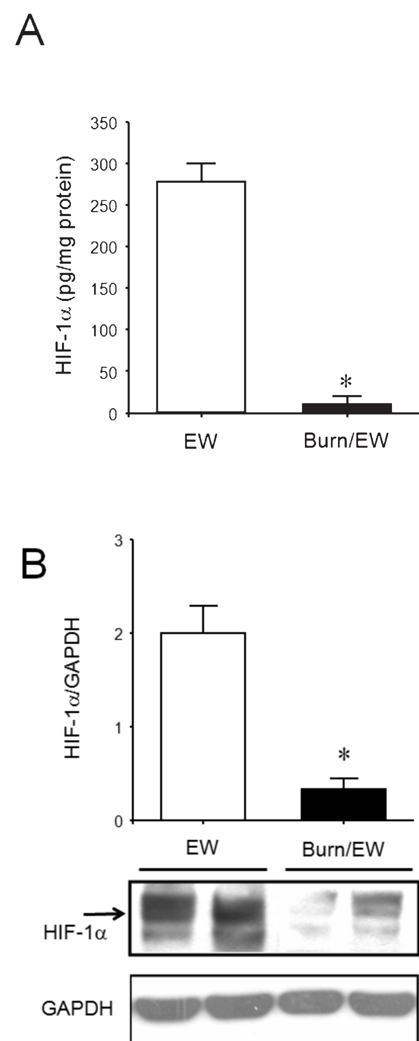

Figure 6. Excisional wound HIF-1α protein expression. At 3 d post-injury excisional wounds were collected from mice receiving an excisional wound only (EW) or a dual injury of thermal injury with excisional wounding (Burn/EW). Wound homogenates were assessed for HIF-1α expression by ELISA (A) and Western blot (B) as described in the Materials and Methods section. Blots are of representative experiments. Arrow indicates band of interest. Data are mean ± SEM; *n* = four mice/group. $^*P < 0.05$ as compared with EW group.

the inflammatory response, it is likely that factors other than the degree of inflammation are involved in the downregulation of HIF-1α in the dual injury group. The markedly suppressed induction of HIF-1α in the dual injury group correlated with slower wound closure. Consistent with our findings, Mace *et al*. (24) have shown that HIF-1α levels are reduced dramatically in wounds of diabetic mice, which displayed markedly reduced healing. Their results, along with the current findings, suggest that HIF-1α function plays a significant role in wound healing and reduced levels of HIF-1α contribute to impaired healing response in a dual injury model of burn and excisional wounding.

In the current study, we limited the analysis of the wound tissues to a single time-point of 3 d post-injury. The rationale for this was based on the observation that the greatest differences between the groups in the systemic response was evident then, and that significant wound closure had occurred in both groups. Furthermore, previous studies indicate that 3 d post-injury is during the inflammatory phase of the healing process, which was of primary interest to the current study (6). Nonetheless, the initial observations made here concerning post-burn wound healing are limited by their descriptive, rather than mechanistic, nature. In light of this, future studies using genetic manipulation of HIF-1α (that is, transgenic animals, siRNA) will be needed to verify a causative relationship between HIF-1α and suppressed wound healing post-burn. In addition, the investigation of other times post-injury is warranted to better correlate the inflammatory response with wound healing derangements.

In conclusion, the current findings indicate that the closure of secondary excisional wounds post-burn was delayed, and associated with a reduced inflammatory response and markedly suppressed expression of HIF-1α at the injury site during the inflammatory phase of the healing process. This suggests that therapeutic regimes that would increase HIF-1α expression early post-burn might improve the healing process and warrant further investigation.

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REFERENCES

1. Spies M, *et al.* (2002) Gene expression analysis in burn wounds of rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283:R918–30.
2. Gosain A, DiPietro LA. (2004) Aging and wound healing. *World J. Surg.* 28:321–6.
3. Gibran NS, Heimbach DM. (2000) Current status of burn wound pathophysiology. *Clin. Plast. Surg.* 27:11–22.
4. Singer AJ, Clark RA. (1999) Cutaneous wound healing. *N. Engl. J. Med.* 341:738–46.
5. Martin P. (1997) Wound healing—aiming for perfect skin regeneration. *Science* 276:75–81.
6. Park JE, Barbul A. (2004) Understanding the role of immune regulation in wound healing. *Am. J. Surg.* 187:11S–6S.
7. Schwacha MG. (2003) Macrophages and post-burn immune dysfunction. *Burns* 29:1–14.
8. Lederer JA, Rodrick ML, Mannick JA. (1999) The effects of injury on the adaptive immune response. *Shock* 11:153–9.
9. Dovi JV, Szpaderska AM, DiPietro LA. (2004) Neutrophil function in the healing wound: adding insult to injury? *Thromb. Haemost.* 92:275–80.
10. Ryan CM, Yarmush ML, Burke JF, Tompkins RG. (1992) Increased gut permeability early after burns correlates with the extent of burn injury. *Crit. Care Med.* 20:1508–12.
11. Herndon DN, Zeigler ST. (1993) Bacterial translocation after thermal injury. *Crit. Care Med.* 21:S50–S54.
12. Eaves-Pyles T, Alexander JW. (1998) Rapid and prolonged impairment of gut barrier function after thermal injury in mice. *Shock* 9:95–100.
13. Wolf SE, *et al.* (1997) Mortality determinants in massive pediatric burns. An analysis of 103 children with > or = 80% TBSA burns (> or = 70% full-thickness). *Ann. Surg.* 225:554–65.
14. Alexander M, Chaudry IH, Schwacha MG. (2002) Relationships between burn size, immunosuppression, and macrophage hyperactivity in a murine model of thermal injury. *Cell. Immunol.* 220:63–9.
15. Szpaderska AM, Zuckerman JD, DiPietro LA. (2003) Differential injury responses in oral mucosal and cutaneous wounds. *J. Dent. Res.* 82:621–6.
16. Frank S, Kampfer H. (2003) Excisional wound healing: an experimental approach. In: *Wound Healing: Methods and Protocols*. DiPietro LA, Burns AL (eds.) Humana Press, Inc, Totowa, NJ, pp. 3–16.
17. Alexander M, Daniel T, Chaudry IH, Choudhry M, Schwacha MG. (2006) T-cells of the γδ T-cell receptor lineage play an important role in the postburn wound healing process. *J. Burn Care Res.* 27:18–25.
18. Daniel T, *et al.* (2007) Regulation of the postburn wound inflammatory response by gammadelta T-cells. *Shock* 28:278–83.
19. Peranzoni E, *et al.* (2007) Role of arginine metabolism in immunity and immunopathology. *Immunobiology* 212:795–812.
20. Malda J, Klein TJ, Upton Z. (2007) The roles of hypoxia in the in vitro engineering of tissues. *Tissue Eng.* 13:2153–62.
21. Dery MA, Michaud MD, Richard DE. (2005) Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. *Int. J. Biochem. Cell. Biol.* 37:535–40.
22. Albina JE, Reichner JS. (2003) Oxygen and the regulation of gene expression in wounds. *Wound Repair Regen.* 11:445–51.
23. Haroon ZA, Raleigh JA, Greenberg CS, Dewhirst MW. (2000) Early wound healing exhibits cytokine surge without evidence of hypoxia. *Ann Surg.* 231:137–47.
24. Mace KA, Yu DH, Paydar KZ, Boudreau N, Young DM. (2007) Sustained expression of Hif-1alpha in the diabetic environment promotes angiogenesis and cutaneous wound repair. *Wound Repair Regen.* 15:636–45.