Published in final edited form as:

Crit Care Med. 2010 January ; 38(1): . doi:10.1097/CCM.0b013e3181b43236.

Insulin increases resistance to burn wound infection-associated sepsis

Gerd G. Gauglitz, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Dermatology and Allergology, Ludwig-Maximilian-University Munich, Munich, Germany.

Tracy E. Toliver-Kinsky, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas

Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, Texas

Felicia N. Williams, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Surgery, University of Texas Medical Branch, Galveston, Texas

Juquan Song, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Surgery, University of Texas Medical Branch, Galveston, Texas

Weihua Cui, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

David N. Herndon, MD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Surgery, University of Texas Medical Branch, Galveston, Texas

Marc G. Jeschke, MD, PhD

Shriners Hospitals for Children, University of Texas Medical Branch, Galveston, Texas

Department of Surgery, University of Texas Medical Branch, Galveston, Texas

Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, Texas

Abstract

Objective: This study was designed to determine the ability of insulin to improve outcome following a *Pseudomonas aeruginosa* wound infection in a rodent model of severe burn injury.

Background: Severe burn injury predisposes patients to burn wound infections that can disseminate, lead to uncontrolled inflammation, and induce septic shock. Whereas insulin administration has been extensively discussed to improve morbidity and mortality in critically ill

patients, the ability of insulin to improve outcomes of severely burned patients with infected burn wounds is not known.

Design: Sprague-Dawley rats.

Setting: University setting.

Intervention: Burn-injured Sprague Dawley rats were randomized into treatment groups that received either saline or insulin. Burn wounds were topically inoculated with a lethal dose of *Pseudomonas aeruginosa* 6 days after injury.

Measurements and Main Results: Survival, systemic dissemination of bacteria, systemic inflammation, and immune activation were examined. Insulin decreased the early inflammatory response to a severe burn injury. Treatment with low doses of insulin following burn injury improved the outcome of rats in response to a lethal burn wound infection. Specifically, survival was improved and systemic dissemination of bacteria from the wound was decreased. Systemic inflammation, indicated by serum interleukin-6 levels, was significantly decreased by insulin treatments after injury. Additionally, insulin treatments were associated with alterations in B and T lymphocyte responses to wound infection.

Conclusions: Although the mechanisms by which insulin improves outcome following a lethal burn wound infection are not known, the data suggest that immunologic responses to infection may be altered by postburn insulin treatments. (Crit Care Med 2010; 38:202–208)

Keywords

burn; inflammation; insulin; wound infection; Interleukin-6

Severe burn trauma is a devastating injury associated with hypermetabolism and a profound catabolic state. Furthermore, it causes substantial perturbations in the immune system through the systemic inflammatory response, often resulting in suboptimal innate and adaptive immune responses (1-3). These changes in the immune system and destruction of the skin barrier increase the burn patient's susceptibility to burn wound infections and may lead to systemic bacterial dissemination, causing sepsis, multiorgan failure, and increased mortality (1, 4, 5). Various patient factors, such as age, immune status, extent of injury, and depth of burn, in combination with microbial factors, such as type and number of organisms, enzyme and toxin production, and motility, determine the likelihood of invasive burn wound infection (6). Advances in topical and systemic antibiotics have improved the treatment of burn-associated infections. However, the subsequent development and use of broad-spectrum antibiotics has propagated the colonization of antibiotic-resistant microorganisms, in particular *Pseudomonas aeruginosa*, one of the most commonly identified organisms in systemic or localized burn wound infections (6-8). Therefore, infectious complications remain a leading cause of death for patients with burns greater than 40% total body surface area (TBSA) (1, 7, 8). Treatment options that attenuate the systemic inflammatory response to burn trauma and enhance host resistance to infection may provide an alternative for severely burned patients.

A recent milestone study found that intensive insulin therapy decreased mortality in critically ill patients. Insulin given at doses to maintain blood glucose below 110 mg/dL prevented the incidence of multiorgan failure and thus improved clinical outcome and rehabilitation (9). However, since strict blood glucose control, in order to maintain normoglycemia, was required to obtain the most clinical benefit, a dialogue has emerged between those who believe that tight glucose control is beneficial for patient outcome and others who fear that high doses of insulin may lead to increased risks for hypoglycemic events and its associated consequences in these patients (9, 10). Studies conducted by our

group further indicated that insulin improves hypermetabolism by decreasing proinflammatory cytokine and hepatic acute-phase proteins production postburn (11, 12). Since insulin has potential as a treatment for reducing hypermetabolism in severely burned patients, it is important to assess the effects of insulin on responses to infection in the susceptible burn patient population. Through attenuation of systemic inflammation, insulin may have immunomodulatory properties that could improve clinical outcome postburn. This study was designed to determine the effects of insulin treatment after burn injury on the host response to infection in a lethal rodent model of burn wound infection.

MATERIALS AND METHODS

Animals

All animal manipulations were approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston. The National Institutes of Health Guidelines for the Care and Use of Experimental Animals were met. Sprague Dawley rats, 325 to 350 grams, were used in these studies and were allowed to acclimate for 1 week before the experiments. Rats were housed in an institutional animal care facility and received a high-protein diet (Ensure, Abbott Laboratories, Abbott Park, IL) and water *ad libitum* throughout the study. Ensure was administered 7 days before the study to adjust the animals to the liquid diet.

Burn Injury

A well-established method was used to induce a full-thickness scald burn in our model (13). Animals were anesthetized with general anesthesia (Ketamine, 40 mg/kg body weight, and Xylazine, 5 mg/kg body weight, both injected intraperitoneally) and received analgesia (Buprenorphine 0.05 mg/kg body weight, injected subcutaneously). The dorsum of the trunk and the abdomen were shaved, and a 60% TBSA burn was administered by placing the animals in a mold exposing defined areas of the skin of the back and abdomen under general anesthesia and analgesia. The mold was placed in 96–98°C water, scalding the back for 10 secs and the abdomen for 2 secs. This method delivers a full-thickness cutaneous burn as confirmed by histologic section. Lactated Ringers solution (40 mL/kg body weight) was administered intraperitoneally immediately after the burn for resuscitation. After burn and resuscitation, animals were observed, given oxygen, and then placed into cages. Unburned (sham) animals received the same treatment except for the scald burn.

Animals were pair-fed with a liquid high-protein high-amino acid nutrition (Ensure, Abbott Laboratories) and water *ad libitum*. Ensure has a caloric distribution of 24% protein, 21% fat, and 55% carbohydrate. All rats were pair-fed according to the caloric intake. The feeding protocol was as follows: 25 calories on the day of burn or sham, 51 calories on the first day postburn, 76 calories on the second, and 101 calories from the third day on after burn. Nutritional intake was the same in all groups.

Burn Wound Infection

Pseudomonas aeruginosa was purchased from the American Type Culture Collection (ATCC #19660). Cultures were grown in tryptic soy broth and diluted in sterile saline for wound inoculation. Six days after burn injury, 6×10^5 colony-forming units (CFU) of *Pseudomonas aeruginosa* were applied to the surface of the wound.

Treatment Groups

Three different studies with several treatment groups were performed. Animals included in the first study received a 60% TBSA burn and animals were injected once daily with either 1 IU/kg body weight of insulin glargine (Insulin Lantus, Sanofi Aventis, Kansas City, MO,

insulin group), or an equal amount of saline subcutaneous (control group, $n = 10$). Sham animals did not receive any burn procedure or treatment. Animals were killed by decapitation without anesthesia 3, 6, 12, 24, and 48 hrs after burn injury and blood was collected immediately thereafter ($n = 10$, each group and time point).

Animals included in the second study were subjected to a 60% TBSA burn and wounds were topically inoculated with 5×10^6 CFU of *Pseudomonas aeruginosa* 6 days later. Randomized groups were treated daily for 28 days with saline or insulin (1 U, 2.5 U, 5 U/kg body weight, sc), $n = 10$ for each group, and rats were monitored daily for survival up to 22 days following wound inoculation. Blood glucose levels were measured daily using a glucometer. Animals included in the third study received a 60% TBSA burn and wounds were topically inoculated with 5×10^6 CFU of *Pseudomonas aeruginosa* 6 days later. Randomized groups were treated daily with saline or 1 U/kg body weight insulin, $n = 10$ for each group. To examine bacterial growth and dissemination, blood and spleens were aseptically harvested 9 days after inoculation and diluted and/or homogenized in sterile saline. Serial dilutions of blood or tissue homogenates were grown overnight on tryptic soy agar for determination of CFU/gram tissue wet weight or mL blood. To examine immune cells, spleens and wound-draining axillary and inguinal lymph nodes were harvested and prepared for flow cytometry staining. Tissues were collected 9 days after inoculation because this duration is the time at which mortalities typically begin to occur.

Cytokine and Serum Glucose Determinations

Blood was collected immediately after harvest and stored on ice until serum preparation. Serum was separated by centrifugation at 4500 rpm for 3 mins at 4°C and stored at -80°C until analyzed. The levels of serum glucose, Interleukin-6 (IL-6), CINC (cytokine-induced neutrophil chemoattractant)-1, CINC-2, and MCP (monocyte chemotactic protein)-1, as markers of systemic inflammation, and serum glucose levels were determined by double-sandwich, enzyme-linked, immunosorbent assays (ELISA-technique, R&D-Systems Inc., Minneapolis, MN) according to the protocol of the manufacturer. Each serum cytokine was determined from standard curves after measurement of optical density at 450 nm.

Flow Cytometry

The spleen and lymph nodes from individual rats were isolated and pooled to prepare single-cell suspensions as previously described (14, 15). Briefly, spleens and lymph nodes were harvested from rats killed by means of an overdose of anesthesia (Ketamine 80 mg/kg body weight, and Xylazine 10 mg/kg body weight, both injected intraperitoneally), placed in 35-mm-diameter dishes containing RPMI-1640 media with 10% fetal bovine serum, and homogenized by means of smashing with the plunger from a 1-mL-capacity syringe. The dispersed spleens and lymph nodes were passed through a 100–2m nylon mesh; erythrocytes were lysed (erythrocyte lysis kit; R&D Systems, Minneapolis, MN) and cells were counted using an automated counter (Coulter Electronics, Fullerton, CA). The cells (quantity, 1×10^7 cells/tube) were placed in polystyrene tubes containing labeling antibodies or isotype controls (quantity, 0.5–1.2 g of antibody per tube) and incubated (temperature, 4°C) for 30 mins.

Cell types were then defined by flow cytometry using well-established lineage-specific markers. The following antibodies were used: fluorescein isothiocyanate-conjugated anti-CD3 (Fitzgerald Industries International, Concord, MA), anti-B220 (Fitzgerald Industries International), anti-Mac387 (Affinity BioReagents, Golden, CO), anti-CD11b (Affinity BioReagents, Golden, CO), anti-granulocytes (BD Bioscience, San Jose, CA), phycoerythrin-conjugated anti-CD44 (Fitzgerald Industries International) and anti-CD71 (Pharmingen-BD, San Jose, CA). The cells were washed with 2 mL of cold phosphate-

buffered saline solution and fixed with 250 μ L of 1% paraformaldehyde. The samples were analyzed using a FACScan flow cytometer (Becton Dickinson, San Diego, CA). Specific staining was determined by comparison with appropriate Ab isotype controls. Immunostaining results were expressed as the percentage of positive cells in spleen and lymph nodes.

Statistical Analysis

For comparisons of data from multiple groups, one-way analysis of variance (ANOVA) was performed followed by the Tukey's multiple comparisons test. Proportion data were analyzed using Fisher's exact test and survival data analyzed using the log rank test. Differences were considered significant at a p value of $\leq .05$. Results are presented as mean $\pm$ SEM. All data were analyzed using GraphPad Prism software (GraphPad Software, Inc, San Diego, CA).

RESULTS

Effects of Insulin on Inflammation in the Early Postburn Period

Burn injury caused a transient but significant increase in blood glucose levels at 3 and 6 hrs postburn in saline-treated rats (3 hrs sham control (sc) vs. burn saline (bs), burn insulin (bi) vs. bs, $p < .001$; 6 hrs sc vs. bs, bi vs. bs, $p < .01$, ANOVA, Tukey's multiple comparison test, Figure 1A). Rats treated with insulin maintained normal blood glucose levels. Soon after burn injury, there were significant alterations in the serum levels of certain cytokines in response to burn alone. Burn injury resulted in augmented serum CINC-1 and CINC-2 concentrations up to 100-fold and ten-fold, respectively, as well as IL-6 and MCP-1 levels up to 160- and four-fold, respectively, over normal values. Daily administration of insulin attenuated the early production of proinflammatory cytokines after burn. Burn injury transiently, but significantly, increased serum IL-6 within the first 3 hrs after burn injury, with levels remaining above normal 48 hrs after injury. Insulin decreased this early IL-6 response to burn injury, with significantly lower levels detected at 6, 12, and 48 hrs postburn, (3 hrs sc vs. bs, bs vs. bi, 6 hrs sc vs. bi, 12 hrs sc vs. bi, 24 hrs sc vs. bi, $p < .01$; 6 hrs sc vs. bs, 12 hrs sc vs. bs, 48 hrs sc vs. bs, bs vs. bi, $p < .001$; 6 hrs bs vs. bi, 12 hrs bs vs. bi, $p < .05$, ANOVA, Tukey's multiple comparison test, Fig. 1B). Similarly, CINC-1 and CINC-2 levels were significantly elevated in response to burn injury alone, and were lowered by insulin administration to significantly lower levels at 3 and 48, and 48 hrs postburn, respectively, (CINC-1: 3 hrs sc vs. bs, sc vs. bi, 6 hrs sc vs. bs, sc vs. bi, 12 hrs sc vs. bs, sc vs. bi, 24 hrs sc vs. bi $p < .001$; 24 hrs bs vs. bi $p < .01$; 3 hrs bs vs. bi $p < .05$; CINC-2: 12 hrs sc vs. bs, 48 hrs sc vs. bs $p < .001$; 12 hrs sc vs. bi, 48 hrs bs vs. bi $p < .01$; 3 hrs bi vs. sc, 6 hrs sc vs. bs $p < .05$, ANOVA, Tukey's Multiple Comparison test, Fig. 1C and D). MCP-1 levels were also elevated after burn injury, and insulin treatment resulted in significantly lower values at 12 and 48 hrs postburn (3 hrs sc vs. bs, bs vs. sc, 6 hrs bi vs. sc, 12 hrs bs vs. sc, 24 hrs bs vs. sc, 48 hrs bs vs. sc, 48 hrs bi vs. sc $p < .001$; 6 hrs bs vs. sc, 24 hrs bi vs. sc $p < .01$; 12 hrs bs vs. bi, 48 hrs bs vs. bi $p < .05$, ANOVA, Tukey's multiple comparison test, Fig. 1E). Serum levels of TNF, IL-1, and IL-10 were not significantly different among the groups (data not shown).

Daily Insulin Administration Improves Survival Following a Lethal Burn Wound Infection

To determine the effects of insulin on survival after sepsis arising from a *Pseudomonas aeruginosa* burn wound infection, 5×10^6 CFUs of *Pseudomonas aeruginosa* were applied to the burn wound 6 days after burn injury. Survival was monitored for 22 days after infection. One unit of insulin significantly increased overall survival ($p < .05$, log rank test), as well as the mean time to mortality compared to saline or 5 U insulin treatment ($p < .05$, ANOVA, Tukey-Kramer test). Additionally, 2.5 U of insulin significantly increased survival compared

with control saline treatment, whereas 5 U of insulin had no effect on survival ($n = 10$ rats per group, Fig. 2A). We found a significant ($p < .001$) correlation between the dose of insulin administered and the mean survival time after wound infection (20.4, 17.6, 14.7 days associated with 1, 2.5 and 5 U insulin, respectively) as shown in Figure 2B. Insulin treatments decreased serum glucose levels throughout the study when compared with saline-treated animals.

Insulin Treatment Decreases Systemic Bacterial Dissemination After Burn Wound Infection

To determine whether improved survival after insulin treatments is caused by the effects of insulin on bacterial clearance mechanisms, bacterial burden in blood and spleen was examined. Nine days following wound inoculation, 5 of 7 (71%) of the saline-treated rats had positive blood cultures (Fig. 3A), whereas only 1 of 8 (13%) of the insulin-treated rats had positive blood cultures, a difference that was statistically significant ($p = .0406$, Fisher's exact test). The mean CFU in the cultures from the saline-treated group was 91 CFU/mL blood, whereas the mean in the insulin group was only 20 CFU/mL blood. In the spleen, 7 of 7 (100%) of the cultures from saline-treated rats were positive, whereas only 3 of 7 (43%) of the cultures from insulin-treated rats were positive. Although not significantly different, the mean CFU/g in the spleens of saline-treated rats (3214 CFU/g) was ~4 times greater than the mean CFU/g in spleens from insulin-treated rats (759 CFU/g, Fig. 3B).

Effects of Insulin Treatment on Systemic Inflammation After Burn Wound Infection

To determine whether insulin treatments also modulate systemic inflammation in response to burn wound infection, proinflammatory cytokine concentrations in plasma were measured after wound inoculation. Nine days after wound inoculation, saline-treated rats had high levels of serum IL-6 (19.3 ± 0.6 ng/mL). Rats treated with insulin had significantly lower levels of serum IL-6 (6.9 ± 0.3 ng/mL), as shown in Figure 4A ($p < .0001$, Student's t test). Plasma levels of IL-1, IL-10, CINC-1, CINC-2 and TNF were not significantly different between treatment groups; however, saline-treated animals displayed considerably elevated levels of serum IL-1 and TNF compared to the insulin-treated rats (data not shown). Insulin treatment markedly reduced serum glucose levels in insulin-treated rats that remained lower at 1 week after wound inoculation when compared with saline-treated rats (Fig. 4B).

Effects of Insulin Treatment on Lymphocyte Activation in Response to Burn Wound Infection

Surface expression of CD71 and CD44, commonly used activation markers (16, 17), was examined on T and B lymphocytes in the spleen and wound-draining lymph nodes 9 days following wound inoculation. CD71 (transferrin receptor) surface expression represents the state of metabolic activity in the cell and must be increased in order to internalize the iron required for T cell DNA synthesis and proliferation (18, 19). In various studies, the CD44 phenotype has been suggested to be a marker of activation, associated with aggressive inflammatory phenomena in various autoimmune models (20, 21). No differences were observed between groups in the percentages of granulocytes, monocytes, or macrophages, nor in the percentages of any leukocytes expressing surface CD44 (data not shown). However, in response to a burn wound infection, there were significant alterations in the number of activated lymphocytes in insulin-treated rats. Specifically, the total number of B220⁺ B cells was significantly lower 9 days following a wound infection in insulin-treated rats (comprising 12.9% of cells), compared with the saline-treated group (28.9% of cells positive for B220). The number of CD71⁺ B cells was significantly decreased in insulin-treated rats, (3.3% and 13.7% of B cells were negative in insulin vs. saline groups, respectively, $p = .0009$), whereas CD71⁺ cells were not significantly altered (Fig. 5). Therefore, the decrease in CD71⁺ B cells resulted in a significant increase in the relative

proportions of activated B cells in the spleen and draining lymph nodes. Whereas 50% of the B cells were activated upon harvest in the saline-treated group, 82% of the B cells were activated in the insulin group ($p = .05$).

On the other hand, the number of CD71⁻ CD3⁺ cells was significantly higher (23.1% vs. 10.9% of T cells in insulin vs. saline groups, respectively, $p = .0212$), and the number of CD71⁺ CD3⁺ cells significantly lower (14.1% and 19.3% of T cells in insulin vs. saline groups, respectively, $p = .0185$), following wound infection in insulin-treated rats. Therefore, only 39% of T cells expressed the CD71 activation marker in insulin-treated rats, whereas 65% of T cells expressed CD71 in the saline-treated group ($p < .05$, Fig. 5).

DISCUSSION

Large surface area burns are frequently associated with burn wound infections that lead to sepsis, multiorgan failure, and increased mortality (4, 5, 7, 22). Intensive insulin therapy was recently found to improve overall morbidity and mortality in critically ill patients by reducing the number of deaths from multiorgan failure with a proven septic focus (9). To date, however, it is currently unknown how and if intensive insulin therapy improves the outcome during critical illness with severe sepsis (10), even though such therapy has been widely advocated (23). Since sepsis associated with *Pseudomonas aeruginosa* infection of burn wounds is a common complication in severely burned patients (22, 24, 25), we developed a clinically relevant model to assess the effect and underlying mechanisms of insulin on survival after a *Pseudomonas aeruginosa* burn wound infection. We found that insulin significantly affected survival in a dose-dependent manner. While one unit of insulin significantly increased overall survival as well as the mean time to mortality compared with saline, 5 units of insulin had no beneficial effect on survival. In our model, we found that both thermal injury and bacterial challenge resulted in a profound increase in blood glucose values. Insulin administration markedly decreased daily blood glucose levels throughout the time period studied when compared with saline-treated animals.

Stress-related hyperglycemia and insulin resistance are common findings in burn and septic patients, and are frequently associated with increased mortality (26, 27). Targeted glycemic control using insulin to improve clinical outcome has been widely demonstrated in various clinical settings (9, 28-32). However, high doses of insulin frequently lead to an increased risk for hypoglycemic events and its associated consequences in critically ill patients (28, 31), a likely cause of death in our 5-unit insulin group. In fact, the very recent NICE-SUGAR trial demonstrated that intensive glucose control may indeed lead to increased mortality among adults in the intensive care unit and suggested glucose levels of 180 mg/dl and below (10).

Having established that low doses of insulin significantly improved survival in our model, we further aimed to elaborate the underlying mechanisms of the effects of insulin on the host response to a *Pseudomonas aeruginosa*-induced lethal burn wound infection. Daily treatments with 1 unit of insulin significantly decreased serum levels of IL-6 after burn wound infection, but did not significantly affect other cytokines measured during this time. IL-6 has been shown to stimulate inflammation by modulating the functional repertoire of mature polymorphonuclear neutrophils, immune cells that appear to be important effectors in the genesis of postinjury inflammation and tissue injury (33). Prolonged and excessive elevations of circulating IL-6 levels in patients after trauma, severe burn, and systemic inflammatory response syndrome (SIRS) have been highly associated with increased morbidity and mortality (34, 35). We have found, in a similar mouse model of burn wound infection, that mortality is associated with higher circulating IL-6 levels in response to infection and higher bacterial burden (36). While IL-6 is important for local defense against

Pseudomonas aeruginosa (37), high systemic levels of IL-6 and higher levels of *Pseudomonas aeruginosa* in the blood suggest that uncontrolled bacterial growth and systemic dissemination may lead to excessive and lethal inflammation. Similar findings of insulin-mediated attenuation of inflammation in other models of trauma and infection suggest that insulin may act as an anti-inflammatory agent to improve clinical outcome (11, 38, 39). Specifically, Dandona and colleagues (40) proposed that insulin may develop its anti-inflammatory effects via reduction of intranuclear NF- κ B and simultaneous stimulation of its inhibitor I κ B. Flow cytometry studies of human leukocytes have shown insulin receptors on lymphocytes, monocytes, and neutrophils (41), and insulin has been found to increase the functions of activated lymphocytes (42, 43) and neutrophils (44) and to enhance the proliferation and differentiation of T cells (43). These data suggest that insulin may have some direct effects on certain immune cell populations. Within this study, we did not observe any effects of insulin therapy on macrophage or granulocyte numbers or activation. However, insulin treatment did appear to affect lymphocytes during the response to a burn wound infection. Unfortunately, the significance of these findings is not clear. Insulin treatment was associated with an increase in the CD71⁻, or nonactivated T-cell population and a decrease in the CD71⁺, or activated T-cell population. Surprisingly, insulin treatment was also associated with a decrease in nonactivated B cells, yet no change in the activated population following infection. Therefore, most (82%) of B cells in the insulin-treated mice were activated following infection. These results are puzzling and cannot currently be explained. However, the specificity of insulin-associated alterations for lymphocyte populations, and not other cells such as macrophages and granulocytes, combined with the decrease in bacterial dissemination after insulin treatment, does suggest that insulin likely has specific effects on immune responses that may increase resistance to burn wound infections. Clearly further studies are warranted to determine the significance of and mechanisms behind these observed effects of insulin on the host response to infection after burn injury.

Numerous alterations in the immune system have been detected following thermal injury in both rodents and humans, including suppressed T-cell proliferation, impaired natural killer cell activity, and macrophage dysfunction (45). The initial inflammatory response to burn injury is often followed by a predominance of anti-inflammatory cytokines (3, 46), and this perturbation of the cytokine milieu is believed to decrease appropriate host responses to subsequent infections. We found that insulin treatments, started immediately postburn, attenuated the early production of several proinflammatory cytokines in a rat model of severe burn injury. Attenuation of early postburn inflammation by insulin may prevent subsequent cytokine perturbations and therefore may help restore cell-mediated immune responses and prevent sepsis in burned patients. In fact, Van den Berghe and colleagues (9) showed that intensive insulin therapy, started immediately upon admission of critically ill patients, prevented several complications, including bloodstream infections. Gray and colleagues (47) found a significant reduction in the incidence of total nosocomial infections in critically ill patients using intensive insulin therapy. The NICE-SUGAR Study Investigators, in contrast, very recently showed that high doses of insulin may lead to increased mortality in these patients (10).

Here, we demonstrated that low-dose insulin administration, started immediately after thermal injury, significantly decreased systemic bacterial dissemination after burn wound infection, suggesting that insulin may help enhance the hosts' resistance to infection.

CONCLUSIONS

In conclusion, treatment of burned rats with low-dose insulin during the immediate period postburn resulted in attenuation of the early burn-induced proinflammatory response. Low-

dose insulin treatment also improved host resistance to a subsequent *Pseudomonas aeruginosa* burn wound infection. Increased survival after infection was associated with improved systemic bacterial clearance, attenuation of the infection-induced proinflammatory response, and altered B and T lymphocyte responses. However, the exact mechanism by which insulin modulates the host response to burn wound infection is not known, and further studies are needed to elaborate specific mechanisms underlying the beneficial effects of low-dose insulin in the prevention of sepsis and mortality after severe burn injury.

Acknowledgments

This study was supported by the American Surgical Association Foundation, Shriners Hospitals for Children 8490, 8660, 8640, 8760, and 9145; NIH R01-GM56687, T32 GM008256, KO1-HL70451, RO1-HD049471; and P50 GM60338, NIDRR H133A020102, and H133A70019.

REFERENCES

1. Murray CK. Infections in burns. *J Trauma*. 2007; 62:S73. [PubMed: 17556989]
2. Schwacha MG. Macrophages and post-burn immune dysfunction. *Burns*. 2003; 29:1–14. [PubMed: 12543039]
3. Sparkes BG. Immunological responses to thermal injury. *Burns*. 1997; 23:106–113. [PubMed: 9177875]
4. Appelgren P, Bjornhagen V, Bragderyd K, et al. A prospective study of infections in burn patients. *Burns*. 2002; 28:39–46. [PubMed: 11834328]
5. Shires GT, Dineen P. Sepsis following burns, trauma, and intra-abdominal infections. *Arch Intern Med*. 1982; 142:2012–2022. [PubMed: 7125790]
6. Pruitt BA Jr, McManus AT, Kim SH, et al. Burn wound infections: Current status. *World J Surg*. 1998; 22:135–145. [PubMed: 9451928]
7. Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms—a five-year study. *Burns*. 2004; 30:241–243. [PubMed: 15082351]
8. Erol S, Altoparlak U, Akcay MN, et al. Changes of microbial flora and wound colonization in burned patients. *Burns*. 2004; 30:357–361. [PubMed: 15145194]
9. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. *N Engl J Med*. 2001; 345:1359–1367. [PubMed: 11794168]
10. Finfer S, Chittock DR, Su SY, et al. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med*. 2009; 360:1283–1297. [PubMed: 19318384]
11. Jeschke MG, Einspanier R, Klein D, et al. Insulin attenuates the systemic inflammatory response to thermal trauma. *Mol Med*. 2002; 8:443–450. [PubMed: 12435855]
12. Wu X, Thomas SJ, Herndon DN, et al. Insulin decreases hepatic acute phase protein levels in severely burned children. *Surgery*. 2004; 135:196–202. [PubMed: 14739855]
13. Herndon DN, Wilmore DW, Mason AD Jr. Development and analysis of a small animal model simulating the human postburn hypermetabolic response. *J Surg Res*. 1978; 25:394–403. [PubMed: 713539]
14. Sherwood ER, Lin CY, Tao W, et al. Beta 2 microglobulin knockout mice are resistant to lethal intraabdominal sepsis. *Am J Respir Crit Care Med*. 2003; 167:1641–1649. [PubMed: 12626348]
15. Toliver-Kinsky TE, Lin CY, Herndon DN, et al. Stimulation of hematopoiesis by the Fms-like tyrosine kinase 3 ligand restores bacterial induction of Th1 cytokines in thermally injured mice. *Infect Immun*. 2003; 71:3058–3067. [PubMed: 12761083]
16. Cortes-Barberena E, Gonzalez-Marquez H, Gomez-Olivares JL, et al. Effects of moderate and severe malnutrition in rats on splenic T lymphocyte subsets and activation assessed by flow cytometry. *Clin Exp Immunol*. 2008; 152:585–592. [PubMed: 18373698]
17. Gridley DS, Baer JR, Cao JD, et al. TNF-alpha gene and proton radiotherapy in an orthotopic brain tumor model. *Int J Oncol*. 2002; 21:251–259. [PubMed: 12118318]

18. Neckers LM, Cossman J. Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proc Natl Acad Sci U S A*. 1983; 80:3494–3498. [PubMed: 6304712]
19. Pighetti GM, Eskew ML, Reddy CC, et al. Selenium and vitamin E deficiency impair transferrin receptor internalization but not IL-2, IL-2 receptor, or transferrin receptor expression. *J Leukoc Biol*. 1998; 63:131–137. [PubMed: 9469483]
20. Brennan FR, Mikecz K, Glant TT, et al. CD44 expression by leucocytes in rheumatoid arthritis and modulation by specific antibody: Implications for lymphocyte adhesion to endothelial cells and synoviocytes in vitro. *Scand J Immunol*. 1997; 45:213–220. [PubMed: 9042434]
21. Haynes BF, Hale LP, Patton KL, et al. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. *Arthritis Rheum*. 1991; 34:1434–1443. [PubMed: 1719988]
22. Rodgers GL, Mortensen J, Fisher MC, et al. Predictors of infectious complications after burn injuries in children. *Pediatr Infect Dis J*. 2000; 19:990–995. [PubMed: 11055602]
23. Dellinger RP, Carlet JM, Masur H, et al. Surviving sepsis campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med*. 2004; 32:858–873. [PubMed: 15090974]
24. Estahbanati HK, Kashani PP, Ghanaatpisheh F. Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. *Burns*. 2002; 28:340–348. [PubMed: 12052372]
25. Schlager T, Sadler J, Weber D, et al. Hospital-acquired infections in pediatric burn patients. *South Med J*. 1994; 87:481–484. [PubMed: 8153775]
26. Marik PE, Raghavan M. Stress-hyperglycemia, insulin and immunomodulation in sepsis. *Intensive Care Med*. 2004; 30:748–756. [PubMed: 14991101]
27. Mikos B, Barkai L. Hyperglycemia and mortality in critically ill children. *Orv Hetil*. 2007; 148:683–688. [PubMed: 17416576]
28. Brunkhorst FM, Engel C, Bloos F, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med*. 2008; 358:125–139. [PubMed: 18184958]
29. Clement S, Braithwaite SS, Magee MF, et al. Management of diabetes and hyperglycemia in hospitals. *Diabetes Care*. 2004; 27:553–591. [PubMed: 14747243]
30. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc*. 2004; 79:992–1000. [PubMed: 15301325]
31. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. *N Engl J Med*. 2006; 354:449–461. [PubMed: 16452557]
32. Vanhorebeek I, Langouche L, Van den Berghe G. Tight blood glucose control with insulin in the ICU: Facts and controversies. *Chest*. 2007; 132:268–278. [PubMed: 17625087]
33. Biffl WL, Moore EE, Moore FA, et al. Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? *Ann Surg*. 1996; 224:647–664. [PubMed: 8916880]
34. Taniguchi T, Koido Y, Aiboshi J, et al. Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. *Crit Care Med*. 1999; 27:1262–1264. [PubMed: 10446817]
35. Yamada Y, Endo S, Inada K. Plasma cytokine levels in patients with severe burn injury—with reference to the relationship between infection and prognosis. *Burns*. 1996; 22:587–593. [PubMed: 8982534]
36. Bohannon J, Cui W, Cox R, et al. Prophylactic treatment with fms-like tyrosine kinase-3 ligand after burn injury enhances global immune responses to infection. *J Immunol*. 2008; 180:3038–3048. [PubMed: 18292526]
37. Cole N, Bao S, Stapleton F, et al. *Pseudomonas aeruginosa* keratitis in IL-6-deficient mice. *Int Arch Allergy Immunol*. 2003; 130:165–172. [PubMed: 12673071]
38. Jeschke MG, Klein D, Bolder U, et al. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology*. 2004; 145:4084–4093. [PubMed: 15192048]
39. Przkora R, Herndon DN, Finnerty CC, et al. Insulin attenuates the cytokine response in a burn wound infection model. *Shock*. 2007; 27:205–208. [PubMed: 17224797]

40. Dandona P, Aljada A, Mohanty P, et al. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: Evidence for an antiinflammatory effect? *J Clin Endocrinol Metab.* 2001; 86:3257–3265. [PubMed: 11443198]
41. Nielsen L, Rontved CM, Nielsen MO, et al. Leukocytes from heifers at different ages express insulin and insulin-like growth factor-1 (IGF-1) receptors. *Domest Anim Endocrinol.* 2003; 25:231–238. [PubMed: 12972378]
42. Brocardo MG, Schillaci R, Galeano A, et al. Early effects of insulin-like growth factor-1 in activated human T lymphocytes. *J Leukoc Biol.* 2001; 70:297–305. [PubMed: 11493623]
43. DeBenedette M, Snow EC. Insulin modulates the interleukin 2 responsiveness of T lymphocytes. *Reg Immunol.* 1990; 3:82–87. [PubMed: 2150918]
44. Spagnoli A, Spadoni GL, Sesti G, et al. Effect of insulin on hydrogen peroxide production by human polymorphonuclear leukocytes. Studies with monoclonal anti-insulin receptor antibodies, and an agonist and an inhibitor of protein kinase C. *Horm Res.* 1995; 43:286–293. [PubMed: 7607614]
45. Toliver-Kinsky TE, Varma TK, Lin CY, et al. Interferon-gamma production is suppressed in thermally injured mice: Decreased production of regulatory cytokines and corresponding receptors. *Shock.* 2002; 18:322–330. [PubMed: 12392275]
46. Schwacha MG, Knoferl MW, Chaudry IH. Does burn wound excision after thermal injury attenuate subsequent macrophage hyperactivity and immunosuppression? *Shock.* 2000; 14:623–628. [PubMed: 11131912]
47. Grey NJ, Perdritz GA. Reduction of nosocomial infections in the surgical intensive-care unit by strict glycemic control. *Endocr Pract.* 2004; 10(Suppl 2):46–52. [PubMed: 15251640]

Figure 1.

Insulin administration significantly attenuates burn-induced proinflammatory cytokine production. Serum levels of glucose (*A*) and cytokine levels of interleukin (*IL*)-6 (*B*), cytokine-induced neutrophil chemoattractant (*CINC*)-1 (*C*), *CINC*-2 (*D*) and monocyte chemotactic protein (*MCP*)-1 (*E*) were determined at various time points postburn. Animals were injected once daily with either 1 IU/kg body weight of insulin or an equal amount of saline subcutaneous. Graphs show means $\pm$ SEM, $n = 9-10$ rats per group. Asterisks denote significantly different from sham control and corresponding insulin groups; #significantly different from sham control; +significantly different from corresponding insulin group, Analysis of variance, Tukey's multiple comparison test.

Figure 2.

Insulin improves survival in a dose-dependent manner after burn wound sepsis. Six days after burn injury, 5×10^6 colony-forming units of *Pseudomonas aeruginosa* were applied to the caudal portion of the burn wound. Randomized groups were treated daily for 28 days with saline or insulin (1 U, 2.5 U, 5 U/kg body weight, sc), $n = 10$ for each group, and rats were monitored daily for survival up to 22 days following wound inoculation. Insulin affected survival in a dose-dependent manner (A). Correlation between the dose of insulin administered and the mean survival time after wound infection (B). Results shown represent 10 different animals per group, as indicated in the text.

Figure 3.

Effect of insulin on systemic bacterial dissemination from burn wounds. Burn wounds were infected with 5×10^6 colony-forming units of *Pseudomonas aeruginosa* 6 days after burn injury. Randomized groups were treated daily with saline or 1 U/kg body weight insulin. Nine days after burn wound inoculation, systemic dissemination of *Pseudomonas aeruginosa* from the burn wound were examined by performing blood (A) and spleen (B) cultures. Points show colony-forming units per mL or gram in individual rats, and numbers indicate the number of positive cultures of the total number of cultures. *Asterisks* denote statistical significance for every comparison between groups, Fisher's exact test. *cfu*, colony-forming units.

Figure 4.

Effects of insulin treatment on systemic inflammation after burn wound infection. Burn wounds were infected with 5×10^6 colony-forming units of *Pseudomonas aeruginosa* 6 days after burn injury. Randomized groups were treated daily with saline or 1 U/kg body weight insulin, $n = 10$ for each group. Nine days after burn wound inoculation, serum levels of Interleukin-6 (A) and glucose (B) were determined. Throughout the figure, histograms depict serum concentrations of interleukin (IL)-6 or glucose at steady state levels. Results shown represent 10 different animals per group, as indicated in the text. Bars represent means; error bars correspond to SEM. Asterisks denote statistical significance.

Figure 5.

Surface marker expression in immune effector cells. Burn wounds were infected 6 days after burn injury. Randomized groups were treated daily with saline or 1 U/kg body weight insulin, $n = 5$ for each group. Spleens and axillary and inguinal lymph nodes from individual rats were harvested 9 days after burn wound inoculation. Single-cell suspensions were prepared and analyzed by flow cytometry after surface staining with antibodies against CD3 (T cells), B220 (B cells), and the activation marker CD71. The y-axis shows the percentages of cells expressing the surface phenotype, and bars indicate the proportions of CD71⁺ (cross-hatched bar) and CD71⁻ (open bars) cells within the specific cell population. Results represent the means $\pm$ SEM from 5 different animals per group. *Significantly different from corresponding control CD71⁻ group; #significantly different from corresponding control CD71⁺ group.