

ESCHARECTOMY has been shown to improve the survival rates and the outcomes in burns. This observational study was conducted to assess the role of escharectomy on the inflammatory mediators in major burns. Seventeen ASA physical status II or status III adult surviving major burn patients were recruited. When the escharectomy was scheduled, a series of blood samples was obtained at -3 and -1 days preoperation, and +1 and +3 post-operation. The changing levels of endotoxin, cytokines, and adhesion molecules were measured with a quantitative sandwich immunoassay. Extensive escharectomy did not appear to have any significant impact on the levels of tumor necrosis factor alpha, interleukin-10, soluble intracellular adhesion molecule-1 and soluble vascular adhesion molecule-1. Meanwhile, endotoxin and E-selectin were significantly decreased after escharectomy. Escharectomy appeared to have a limited immunomodulatory effect on the inflammatory mediators in systemic inflammatory responses induced by major burns. This is probably related to the timing and extent of surgery, and the complex nature of burn-related inflammation.

Key words: E-selectin, Interleukin-10, soluble intercellular adhesion molecule-1, Thermal injury, Tumor necrosis factor alpha, Soluble vascular adhesion molecule-1, Burn wound excision

The limited immunomodulatory effects of escharectomy on the kinetics of endotoxin, cytokines, and adhesion molecules in major burns

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Introduction

The patho-physiological events following the immense tissue damage of major burn injury include the surface effects of the heat on superficial tissue, but also should be viewed as an acute systemic illness,^{1,2} initiated by a number of processes such as the development of ischemia-reperfusion injury related to burn shock and resuscitation as well as the presence of endotoxemia.³ Major burns produce both local and systemic disturbances due to the release of inflammatory mediators, depending upon the type and extent of burn and the time elapsed since the injury.⁴

The acute inflammatory response results from an integration of a complex cascade of events, including vasodilation, increased vascular permeability, and exudation of fluid and plasma protein. The rapid overproduction of inflammatory active substances, leukocyte activation, activation of endothelial cells, and expression of adhesion molecules and induction of nitric oxide synthase all lead to systemic inflammatory response syndrome (SIRS), resulting in progressive multi-organ failure and death.⁵⁻⁷

Many experimental and clinical studies have demonstrated a pronounced immunosuppression following major burn injury.⁸ Since burn eschar is

thought to be the source of denatured proteins, mediators, and toxins, which cause immunosuppression and infection, prompt excision of burn eschar could be an effective way to avoid absorption of immunosuppressive substances.⁹ Removing the dead devitalized tissue and grafting would not only remove the source of infection, but also decrease the release of inflammatory mediators, and improve survival, prevent postburn immunosuppression and maintain skin barrier function.¹⁰ Experimentally, although some specific parameters of immunity may be improved by early excision and wound closure,¹¹ little defined physiologic benefit has been proved.

A variety of inflammatory mediators such as endotoxin, complement fragments, cytokines, adhesion molecules and lipid mediators are generated shortly after the burn injury at the site of the initial lesion and serve to recruit inflammatory cells at the later stages. In the present study, we measured the dynamic changes of the levels of endotoxin, proinflammatory and anti-inflammatory cytokines, and soluble adhesion molecules, and compared their relations before and after extensive escharectomy. Attempts were made to better understand the immunological role of extensive early escharectomy in preventing SIRS.

Materials and methods

The protocol was reviewed and approved by the institutional ethics committee for human study, and written informed consent was obtained before the study from each patient or legal guardian if mentally incompetent.

When a major burn patient with possible candidacy for the study arrived in the emergency department, the anesthesia department was notified for the potential study subject identification. As the patients were scheduled for the escharectomy, the primary investigator was informed at least 3 days before.

At this time, patients were selected based upon their clinical status, and those with inhalational injury, SIRS, sepsis, septic shock, adult respiratory distress syndrome and multiple organ dysfunction syndromes were included. The guidelines were based on the criteria of the College of Chest Physicians/Society of Critical Care Medicine.^{12,13}

Patients who suffered from any type of immune deficiency syndrome, had taken any immune therapy, steroids or anticancer drug, any hormonal medications or who had immune system disease were excluded from the study. Since it has been well known that non-survivors have an exponential increase in the inflammatory mediators, concerns were raised that those results might falsely elevate the levels. Therefore, samples from those patients were excluded in the final analysis.

On recruitment, an arterial line for multiple sampling was placed in either the upper or lower extremity, in the intensive care unit, and locked with heparin. Serial collections were performed four times between 8:00 and 9:00 a.m. at 72 and 24 h before and 24 and 72 h after the surgery. Three milliliters of blood specimens were collected in endotoxin-free ethylenediamine tetraacetic acid-containing blood specimen tubes (BD Vacutainer™; BD Vacutainer Systems, Plymouth, UK). These were immediately centrifuged at 3000 rpm for 60 sec within 30 min of blood sampling, and platelet-rich plasma was obtained and stored in aliquots at -70°C until the later combined assay. These samples were kept in the refrigerator until each individual patient's eventual outcome was determined. All the samples were analyzed at one time, approximately 3 months after the collections were completed.

Anesthesia was induced by intravenous sodium thiopental, vecuronium and fentanyl, and was maintained by continuous infusion of fentanyl, nitrous oxide and oxygen, and sevoflurane. Intermittent boluses of vecuronium provided muscle relaxation.

Endotoxin levels were measured by the chromogenic limulus amoebocyte lysate (LAL) test (QCL 1000®; Biowhittaker, East Rutherford, NJ, USA), which is a quantitative test for Gram-negative bacter-

ial endotoxin. A sample is mixed with the LAL supplied in the test kit and incubated at 37°C for 10 min. A substrate solution is then mixed with the LAL sample and incubated at 37°C for an additional 6 min. The reaction is stopped with stop reagent. If endotoxin is present in the sample, a yellow color will develop. The absorbance of the sample can be determined spectrophotometrically at 405–410 nm. Since this absorbance is in direct proportion to the amount of endotoxin present, the concentration of endotoxin can be calculated from a standard curve.

Cytokines of tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10) were measured with quantitative sandwich enzyme-linked immunosorbent assay (ELISA) test kits (Quantikine®; R & D systems, Inc., Minneapolis, MN, USA) according to instructions given by the manufacturer and optical densitometry. These assays did not measure biological activity, but rather quantitative assessment of immunoreactivity is provided. The minimum detectable doses of TNF- α and IL-10 with these kits were 4 and 3.9 pg/ml.

Three adhesion molecules of soluble intracellular adhesion molecule-1 (sICAM-1), soluble vascular adhesion molecule-1 (sVCAM-1), and E-selectin were all measured similarly with the same quantitative sandwich ELISA test kit and optical densitometry. The minimum detectable doses of sICAM-1, sVCAM-1 and E-selectin were 0.35, 0.6, and 0.5 pg/ml, respectively.

Measured parameters included the demographic and surgical data such as age, gender, height and weight, burn type and percent of total body surface area, as well as American Society of Anesthesiologists physical status classification.

The SPSS program (version 10.01; SPSS Inc., Chicago, IL, USA) was used for statistical analysis and all values are expressed as mean \pm standard deviation. Demographic and surgical data were summarized by descriptive analysis. The level of changes of endotoxin, cytokines, adhesion molecules and E-selectin were individually determined with repeated-measures of analysis of variance (ANOVA) with Bonferroni's correction. Each patient served as their own control, values were compared before and after the escharectomy. $p < 0.05$ was considered statistically significant. Pearson's correlations were performed to delineate the relationship between the various inflammatory mediators.

Results

A total of 25 patients were recruited, and from these three expired within 1 week after the escharectomy and another five suffered from major complications described as follows before or after surgery. Those samples were exempted from final analysis. The

remainder was 17 major burn patients with a total body surface area of 25% or more, who survived more than 3 months after blood sampling, and from whom blood specimens could be repeatedly obtained. Every other day samples from those patients were finally analyzed with ELISA.

The patients' characteristics and surgical data are summarized in Table 1. Most suffered from extensive major burns. The removal of dead devitalized tissue was performed approximately within 1 week.

Figure 1 shows the changes of endotoxin levels perioperatively. Statistically significant differences were noted before and after escharectomy.

Figure 2 shows the changes of TNF- α and IL-10. In both groups, only day +1 showed a significant decrease in their levels. These were increased at +3 days, following the increasing trend again. Interestingly, the level would have been thought to be there anyway even without the scheduled escharectomy.

Figure 3 shows the changes of adhesion molecules. sICAM-1 showed a continued increasing trend regardless of escharectomy compared with the baseline. In contrast, sVCAM-1 decreased even before the surgery, and remained as such during the rest of study period regardless of the extensive escharectomy. It was even slightly increased after the procedure. E-selectin had a statistically meaningful decrease after the extensive escharectomy and remained at a lower level compared with the baseline at +3 days.

Pearson's correlation analysis revealed statistically significant high correlations between endotoxin and E-selectin, as well as between TNF- α and IL-10.

Discussion

In this study, we have shown that the immune modulatory effect of escharectomy on the kinetics of endotoxin, cytokines, and adhesion molecules in major burns appeared to be limited, in that it did not change that much over a matter of days. In essence, our data did not demonstrate that the removal of the

Table 1. Patient characteristics, American Society of Anesthesiologists (ASA) physical status classification, and burn data

Characteristic	Value
Age (years)	40.9 \pm 9.3
Sex (male/female)	12/5
Weight (kg)	60.5 \pm 4.8
Height (cm)	160.7 \pm 5.6
ASA class (II/III)	3/14
Total body surface area (%)	42.9 \pm 17.0
Elapsed days since initial injury until escharectomy	5.3 \pm 1.7

Values presented as mean \pm standard deviation.

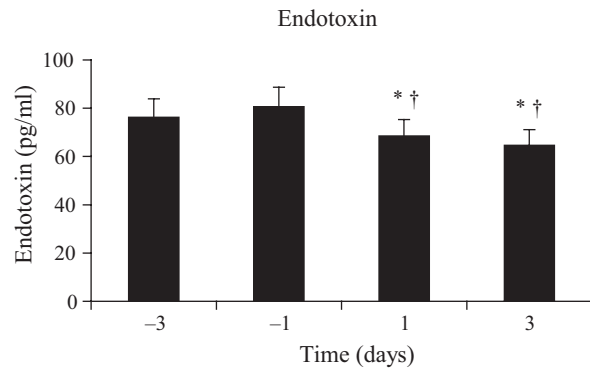


FIG. 1. Bar graph illustrating the changing levels of endotoxin before and after the extensive escharectomy in major burns (repeated-measures ANOVA, * $p < 0.05$ when -3 days compared with -1, +1 and +3 days, † $p < 0.05$ when -1 day compared with +1 and +3 days).

burn has much of a profound effect. Although we found that values of endotoxin and E-selectin decreased significantly after escharectomy, it probably has no clinical significance. In spite of these findings, it cannot be precluded that early removal of the burn does not affect the patient's ability to survive or resist infection.

A number of reasons should be considered to explain the limited, minimal responses to escharectomy in our study. The patient's selection issue should be considered at first. Only the samples taken from survivors were used for the final analysis. Perhaps the

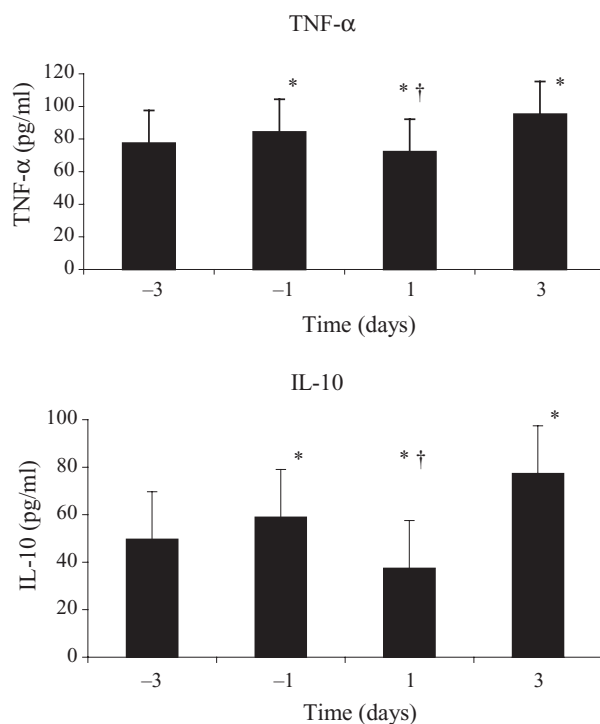


FIG. 2. Bar graphs illustrating the changes of TNF- α and IL-10 before and after extensive escharectomy in major burns (repeated-measures ANOVA, * $p < 0.05$ when -3 days compared with -1, +1 and +3 days, † $p < 0.05$ when +1 day compared with -1 and +3 days).

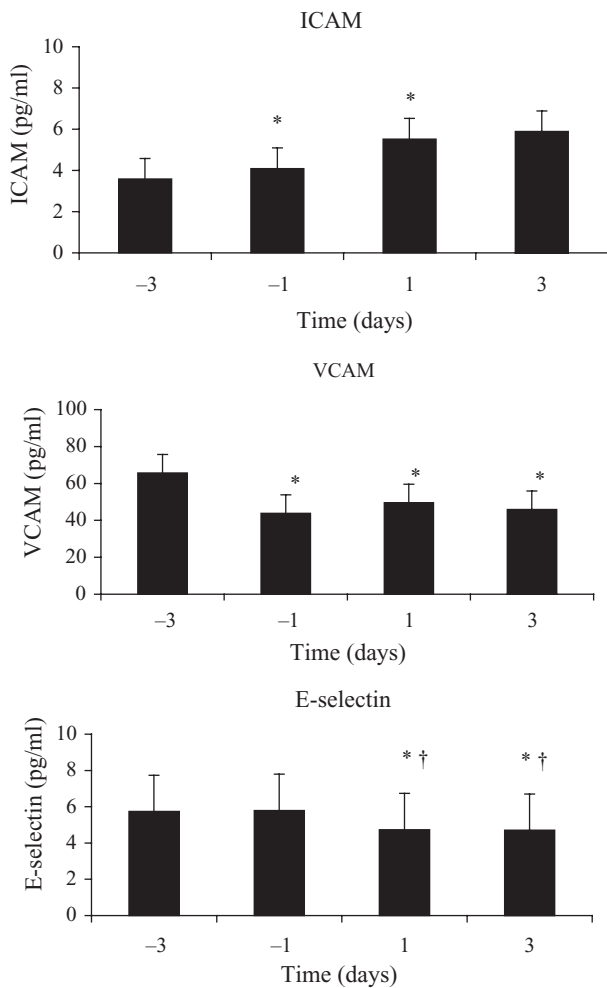


FIG. 3. Bar graphs illustrating the changes of sICAM-1, sVCAM-1, and E-selectin before and after extensive escharectomy in major burns (repeated-measures ANOVA, * $p < 0.05$ when -3 days compared with -1, +1 and +3 days, † $p < 0.05$ when +1 day compared with -1 and +3 days).

patients were not stressed enough. Possibly, the burns were too small. The immune responses for the non-survivors have been shown to be dramatic.¹⁴ We intentionally excluded these patients since their results could have falsely elevated the results and may have masked the true effect of escharectomy. It was not surprising that the changing trend was somewhat less than impressive.

Second, the timing and extent of surgery might have been another factor. We considered a few days before and after the burn excision. This may not be enough time to see a difference. It frequently takes more time for inflammatory mediators and the stress response to resolve. In our burn center, it is customary to perform escharectomy at approximately 5 days (3–7 days) after initial thermal injury and the burn shock stage. Furthermore, removing dead and devitalized tissue is limited and partial, and carried out in multiple staged operations. It had been previously suggested that prompt escharectomy could reduce the production and release of inflam-

matory cytokines, and lessen the myocardial injury induced by uncontrolled inflammatory response. Escharectomy *en masse* during the shock stage could weaken the ability of burn sera to induce the secretion of inflammatory cytokines by macrophages. It was therefore beneficial for the prevention of the development of postburn systemic inflammatory response syndrome.¹¹ The likelihood is high that when the removal of dead devitalized tissue is delayed to after the burn shock stage, the systemic release of inflammatory mediators from the eschar has already been initiated and settled in the systemic circulation. Accordingly, escharectomy performed 5 days after the burn trauma as in our study would not significantly affect the levels of inflammatory mediators.

Clinically and immunologically, many factors other than escharectomy should help burn patients. The use of antibiotics, daily wound dressing, immediate fluid resuscitation, adequate nutritional support, management of pulmonary complications and good covering of the burn wounds have all contributed to the recuperation of burn patients and improve immunologically. Postoperatively, the patients still have donor sites that need to heal, so in essence the wound is not closed.

Third, the general properties of cytokines and other inflammatory mediators are very vulnerable and relatively non-specific, such that production is dependent on the status of the factories, especially macrophages. The potential for a self-perpetuating process, because of a lack of a negative feedback loop, can explain the progression of inflammation, sepsis and organ failure, even in the absence of continuing injury or infection. The level of circulating cytokines often does not necessarily correlate with the degree of clinical illness and tissue activity. This is because the signal to release the secondary mediators is initiated by the cytokines, and the continued presence of the signal is no longer required. In addition, the action of the cytokines is either at the cell surface receptor or inside the cell, and circulating levels often do not reflect the intracellular levels. The patients also have much more pain postoperatively, which may also turn on cytokine expression. Measuring the cytokines after total healing may make a difference. For these reasons, it is not surprising that circulating levels of many of the cytokines often do not necessarily reflect the clinical status of critically ill patients, including those with sepsis and burns.¹⁵

Fourth, a variety of inflammatory mediators in trauma, sepsis and burns interact in a complex manner to produce the disease state. Multiple alterations in inflammatory and immunologic function have been demonstrated in clinical and experimental situations after trauma and hemorrhage; in particular, the activation of various humoral (e.g. complement, coagulation) and cellular systems (neutrophils,

endothelial cells, macrophages).¹⁶ As a consequence of this activation process, there is synthesis, expression and release of numerous mediators (toxic oxygen species, proteolytic enzymes, adhesion molecules, cytokines), which may produce a generalized inflammation and tissue damage in the body. Mediators are responsible for ongoing interactions of different cell types and for amplification effects through their networks and feedback cycles, finally leading to sustained inflammation and multiple organ damage in the body. In the setting of trauma and shock, many activators including bacterial as well as non-bacterial factors may be present that induce local and systemic inflammatory responses. Although the potential role of bacteria and endotoxin translocation and its clinical relevance remains controversial, many lines of evidence support the concept that the gut may be the reservoir for systemic sepsis and subsequent multiple-organ failure in a number of pathophysiologic states.¹⁷ Many of the problems that burn doctors deal with come with a secondary infection, not with the initial insult. If we measured the levels after sepsis then we might find a more pronounced difference. Most people are not willing to wait until patients become sick before they remove the burn. We all remove it within 1 week, so our patients do pretty well.

Endotoxin

In burns, endotoxin values were high in the poor outcome group, serving as the trigger for evolution of burn-related pathology.¹⁸ Escharectomy during burn shock was shown to effectively eliminate the effects of the endotoxemia, thereby preventing the accentuated response to endotoxin that is seen when the burn wound is left intact, even if it is uninfected.¹⁹

Cytokines

There is a growing interest in determining the role of TNF- α in burns since an intense immuno-inflammatory reaction is expected to take place in septic shock, severe sepsis and trauma, including major burns patients. Many suggest that the beneficial effects of early extensive burn wound excision and grafting are likely to be related to the normalization of increased macrophage TNF- α production, the amelioration of hypermetabolism with sepsis, as well as the maintenance of skin barrier function.^{9,20,21}

We were able to show a temporary TNF- α decrease to escharectomy, which returned to its increasing trend again within 72 h at a much higher level than the baseline. This is probably due to the relatively non-specific nature of cytokine response to stress. Unless the devitalized dead tissue is to be removed in *en masse* at the early stage of injury, its effect in TNF- α would probably be minimal.¹¹ In addition,

clinical deterioration after burn wound dressing or debridement may be related to predominantly the systemic release of IL-6, instead of TNF- α .²²

IL-10 exerts a number of anti-inflammatory effects, including regulation and inhibition of the synthesis of the inflammatory cytokines and immune responses. The production of IL-10 seems to be predominantly stimulated by TNF- α . Hence, they will be likely to have similar kinetic profiles with TNF- α . Increased levels of IL-10 have been detected during cardiac surgery after an increase in the proinflammatory cytokines, such as TNF- α , and may represent an endogenous response to limit the inflammatory response.²³

Adhesion molecules

The importance of early escharectomy during the burn shock period has been previously emphasized by the prevention of expression of adhesion molecules including sICAM-1 and E-selectin mRNA and SIRS.¹¹ Our results showed that sICAM-1 increased regardless of the performance of escharectomy. This can be attributed to the time and extent of surgery, in that ours was a partial, delayed procedure although it was extensive.

After a wound revision in severe burns, the patients may show signs of sepsis, a transient endotoxemia, and increased expression of the β_2 integrin CD11b, as well as elevated plasma levels of soluble E-selectin, indicating its close association to systemic inflammation.²⁴ On the contrary to sICAM-1, however, E-selectin is a late responder of the inflammation, which might explain the better response to escharectomy performed later stage in our patients.²⁵

In conclusion, massive tissue trauma causes immediate activation of endotoxin, selected chemokines and adhesion molecules within minutes of the injury, which will then persist depending on the type and severity of the injury for a substantial length of time. The timing and extent of removal of dead devitalized tissue is the most influencing factor in modulating immune mediators. Therefore, the importance of early *en masse* burn wound excision during the burn shock stage should be clinically emphasized in major burn patients to maximize its benefits, such as decreased susceptibility to complications or improvement of outcome.

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