Early burn excision attenuates the postburn lung and systemic response to endotoxin.

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The lung and systemic physiologic response to endotoxin is markedly accentuated in the presence of a body burn. Our purpose was to determine whether early burn excision and closure would decrease this response. We compared the endotoxin (2 μg/kg)-induced response in 10 adult sheep with lung and soft-tissue lymph fistulas 3 days after a 15% total-body surface full-thickness burn that was excised immediately with that of sheep without burn excision and nonburned sheep. No infection was present in the burn wound. Early excision prevented the ongoing postburn lipid peroxidation and lung inflammation seen 3 days after burn before endotoxemia in animals with 15% total body surface burn wound not excised. Sheep that underwent excision demonstrated significantly less pulmonary hypertension and hypoxia after endotoxin than did either endotoxin-treated and intact burned sheep or endotoxin-treated nonburned sheep. Lung inflammatory changes as determined by neutrophil content of lung tissue and the increase in lung tissue malondialdehyde in the group that underwent burn excision after endotoxin were comparable to those seen with endotoxin alone, as was the lung lymph-flow response. Also, the systemic response was nearly identical to that seen with endotoxin alone with no increase in soft-tissue permeability as measured by lymph flow. Oxygen consumption (VO₂) remained unchanged from baseline. In contrast, VO₂ doubled in burn-intact animals initially after endotoxin, after which VO₂ decreased to levels below baseline. An increase in soft-tissue vascular permeability was also noted. We can conclude that early burn excision and closure prevent the accentuated response to endotoxin that is seen when the burn wound is left intact, even if it is uninjected. (SURGERY 1990;108:28-35.)

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The response to infection after burn injury appears to be markedly accentuated compared with that seen in nonburned patients. Mortality rates for single organ failure, the most common being lung, approach 100%. Systemic hemodynamic instability, in the presence of lung dysfunction, appears to be the major cause of death in the postburn period. The lung therefore appears to be the major cause of death in the postburn period. The lung therefore appears to play a key role in the sepsis response. We have demonstrated that endotoxemia produces marked lung and systemic physiologic abnormalities in adult sheep in the presence of a 3-day-old uninjured burn, compared with endotoxin alone. Oxidant-induced tissue damage, as reflected in the lipid peroxidation process, is considered a major factor in the sepsis injury. Inflammatory cells are a likely source of the oxidants. Ongoing oxidant-induced lipid peroxidation is well described after burns in animals and humans even before any septic insult, as evidenced by increased circulating and tissue lipid peroxidation. Lipid peroxidation has been reported to increase the sensitivity of tissues to a subsequent oxidant injury. The burn wound would be the logical stimulus for the continued lipid peroxidation, as well as a source of other mediators that would accentuate a subsequent septic insult. Intense lung inflammation is also evident after a skin burn, which could amplify a second insult because the cells could be primed by the initial burn. Early burn excision and wound closure do not prevent initial lung neutrophil sequestration, indicating that this process occurs almost immediately after burn injury. However, early excision does prevent postburn hypermetabolism, indicating the importance of the
wound on this inflammation-induced process. However, either the lung or the burn wound could be responsible for the later accentuated response to endotoxin. The lung could also be the source of the ongoing oxidant response seen in the postburn period.

These issues are of considerable clinical importance because the burn wound can be removed early after injury. At present the primary indication for this procedure is to remove a potential source of local infection and decrease the hospital stay. If the burn wound was also an amplifier of the response to any infection, burn excision would need to be performed within the first several days to prevent an accentuated response from any subsequent infection such as pulmonary infection after inhalation.

Our objectives in this study were twofold. First, we wanted to determine whether the burn wound itself was responsible for the increased physiologic changes seen with an endotoxin insult in the postburn period, excising and completely closing the wound 3 hours after injury. Second, we wanted to determine the effect of early wound removal on the lipid peroxidation process seen in the postburn period in an effort to correlate the physiologic and biochemical changes. We used a 15% total-body surface full-thickness burn as used in prior studies because this degree of burn is sufficient to produce the systemic oxidant response and the accentuated endotoxin response. Yet this injury is small enough to be maintained infection free and to be totally excised.

We gave the endotoxin 3 days after burn injury because the accentuated endotoxin response appears maximal at this time and both burn wound and postburn lung inflammation are present. In addition, the postburn hypermetabolic state has peaked at this time. Lipid peroxidation was monitored by circulating conjugated dienes and lung tissue malondialdehyde (MDA) content, both well-recognized parameters. Some animals were killed 3 days after excision before endotoxin to determine the effect of excision alone on the postburn lipid peroxidation process. Other sheep were killed 6 hours after endotoxin to determine the effect on the subsequent endotoxin response. The burn wound was demonstrated not to be infected at the time of endotoxia by use of quantitative cultures.

**METHODS**

Ten adult yearling sheep, specifically bred for research, weighing 45 to 55 kg, were studied. Prefemoral lymph fistulas were produced to monitor transvascular fluid flux and mediator release from systemic tissues, and lung lymph fistulas were produced to monitor similar parameters in the lung. Vascular catheters were also placed at this time. Animals were allowed to recuperate for 3 days after the surgical

**Fig. 1.** The pulmonary physiologic response to endotoxia presented as a group mean ± SD 3 days after a burn that was immediately excised (O) is compared with a burn injury not excised (•) and unburned sheep (□). There were no differences noted between preburn and 3-day postburn baseline values. The response and degree of hypertension and hypoxia were significantly less with the group with excised burns after endotoxin compared with the group with intact burns and endotoxin given to unburned sheep. PaO₂, Partial pressure of oxygen in arterial blood; L/P ratio, lymph/plasma ratio.
Table I. Lung histologic changes

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Vascular congestion</th>
<th>Neutrophils per HPF</th>
<th>Mononuclear cells per HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>14</td>
<td>0 ± 1</td>
<td>2 ± 1.7</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>Endotoxin alone*</td>
<td>14</td>
<td>2 ± 0.6†</td>
<td>10 ± 1.5†</td>
<td>29 ± 10†</td>
</tr>
<tr>
<td>Endotoxin 3 days after burn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn intact*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before endotoxin</td>
<td>6</td>
<td>1 ± 1.5*</td>
<td>5 ± 2.2†</td>
<td>27 ± 14†</td>
</tr>
<tr>
<td>After endotoxin (6 hr)</td>
<td>6</td>
<td>3 ± 0.6†</td>
<td>11 ± 3.4†</td>
<td>35 ± 12†</td>
</tr>
<tr>
<td>Burn (excised)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before endotoxin</td>
<td>4</td>
<td>0 ± 1</td>
<td>3 ± 1.4</td>
<td>23 ± 11</td>
</tr>
<tr>
<td>After endotoxin (6 hr)</td>
<td>6</td>
<td>2 ± 0.7†</td>
<td>10 ± 2.6†</td>
<td>31 ± 9†</td>
</tr>
</tbody>
</table>

HPF, High-power field.
*Described previously.4-6
†Significantly different from control, p < 0.05.

procedure. Baseline preburn measurements were then obtained.

Burn injury. Three days after the initial lymphatic preparation, a burn injury was produced. The protocol was approved by the animal care committee of the institution. Animals were anesthetized with 1% halothane, 20% nitrous oxide, and 80% oxygen. A tidal volume of 10 ml/kg at a rate of 10 to 14 per minute was used to maintain a normal partial pressure of carbon dioxide in arterial blood. The burn was produced with water at a temperature of 95°C, which was poured on the hide of one entire flank until the hide was uniformly blanched white over 15% of total body surface. Surface-area measurements were calculated according to the method of Bennett.18 We previously determined this degree of injury to be a full-thickness burn and therefore a relatively anesthetic wound. Care was taken to avoid burn injury to the abdomen. The exposure time of hot water necessary to produce this depth of burn is 10 to 15 seconds. The burn was placed over the distribution of the cannulated prefemoral lymphatic vessel. Volume resuscitation was than begun to maintain filling pressures and cardiac output at baseline levels by infusion of lactated Ringer's solution during the first 24 hours. Beginning at 2 hours, the burned hide was removed down to the fascia. Additional lactated Ringer's solution was also infused during the procedure to replace blood lost; replacement was an estimated three times the blood volume loss. Fluids were warmed to 90°F. The animals were also warmed externally with a heat lamp to avoid hypothermia.

A piece of hide equal to the size of the burn was removed from a donor animal during this period. The donor animal was then killed. The hide was defatted and then sutured to the edge of the excised wound and the underlying fascia, and a soft bulky compression dressing was applied. The procedure required approximately 1 hour. Animals were then awakened and returned to their cages. The animals were maintained in a temperature-controlled environment in which room temperature was kept between 70°F and 82°F, which is well within the range of thermal neutrality for sheep.

Meperidine (Demerol, 25 to 50 mg) was administered intramuscularly every 6 to 8 hours if the animals appeared uncomfortable as evidenced by decreased activity, tachypnea, or lethargy. Animals were given cefazolin, 1 gm, before the anesthetic and three classes during the next 24 hours. Food and water were also provided, beginning within 2 hours of surgery. The compression dressing was removed in 24 hours, and the suture line was inspected and covered with povidone-iodine (Betadine) ointment.

Study protocol. Animals were studied in the awake state on the third postburn day. After a 3- to 4-hour baseline was obtained, four animals were killed and postmortem analysis was performed. Six animals were given a dose of Escherichia coli endotoxin, 2 µg/kg intravenously, over 30 minutes. All endotoxin was from a standard lot (No. 652888; Difco Laboratories Inc., Detroit, Mich.). Lactated Ringer's solution was infused to maintain a constant pulmonary artery wedge pressure. The six sheep were followed up for 6 hours and then killed. Physiologic, biochemical, and histologic data were compared with those obtained from control animals and endotoxin alone, burn alone, and burn plus endotoxin-treated animals obtained from our studies during the past 6 months. Some of the data from these animals have recently been reported but not yet published.4-6 All animals were the same breed, age, and sex, came from the same source, and were screened for any illness. We therefore believe that comparison of current animal data with those obtained during the past
Physiologic measurements. Aortic, central venous, pulmonary artery, and pulmonary wedge pressures were recorded (model 1 Cl-58 polygraph; Gilson Medical Electronics, Inc., Middleton, Wis.) by means of calibrated pressure transducers leveled at the point of the shoulder, which we considered to be the level of the left atrium. Cardiac output determinations were measured by the thermodilution method (model COM-1; American Edwards Laboratories, Santa Ana, Calif.). Also obtained were arterial and mixed venous blood gases and body temperature. Blood O2 saturation was calculated according to the Fick equation. Lymph flow (QL) was measured every 30 minutes during the study period.

Biochemical measurements. Blood samples were obtained for white cell counts and differentials. Heparinized samples of plasma from the aorta and pulmonary artery and lymph were collected at hourly time periods during the study period for subsequent measurements of hemoglobin total proteins and lipid peroxides as conjugated dienes. The conjugated diene content of lymph and plasma was measured according to the method described by Recknagel and Glende.19 Conjugated diene content, a measurement of lipid peroxidation, was read at an optical density of 233 nm in a spectrophotometer (Gilford 260; Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

Postmortem analysis. Physiologic, biochemical, and histologic data for the study animals were compared with the previously studied burned and nonburned sheep given endotoxin. A final blood sample for determination of lipid peroxides was obtained. Animals were then injected with ketamine, 15 ml/kg, and after onset of anesthesia with an overdose of a 10% KCl solution. The lungs were inflated to a lung volume of 15 ml/kg and rapidly removed and inspected. The right lung was removed for the measurement of extravascular lung water.20 A biopsy specimen was also obtained for histologic examination. Sections were stained with hematoxylin and eosin stain. The left lung was immediately perfused through the pulmonary artery with cold (0° to 4° C) lactated Ringer’s solution containing meclofenamate (10 mg/dl) to remove intravascular blood and inhibit the postmortem oxidation of any free arachidonic acid, which could add to the MDA content.

Lung histologic findings were compared with those of control lungs in both a qualitative and quantitative fashion. Vascular congestion was ranked 0 if no congestion was present, +1 to +2 for mild to moderate congestion, +3 for severe congestion but no alveolar hemorrhage, and +4 if alveolar hemorrhage was also present. Polymorphonuclear leukocytes in total lung tissue were counted in 15 high-power fields (X1000) per specimen and the average of the fields was recorded.

Lung and tissue lipid peroxidation measured as MDA was also determined. Detection of MDA production was done colorimetrically with the thiobarbituric acid reaction test.14 Pieces of lung tissue, 2 cm3, were removed from the upper and middle portions of the left lower lobe, with care being taken to remove any large bronchioles or vessels from the specimens. The tissue was then gently blotted free of excess water before being homogenized. The level of lipid peroxides was expressed as moles of lipid peroxides per gram of lung tissue.

Quantitative cultures of full-thickness donor hide eschar biopsy specimens were obtained when the animals were killed, as well as cultures of the wound beneath the hide.21

### Table II. Postmortem lung changes

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Lung tissue MDA (nmol/gm)</th>
<th>Lung water (ml H2O/gm dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>6</td>
<td>44 ± 8</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>After endotoxin alone*</td>
<td>6</td>
<td>79 ± 7†</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Endotoxin 3 days after burn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn intact*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before endotoxin</td>
<td>4</td>
<td>64 ± 14†</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>After endotoxin (6 hr)</td>
<td>4</td>
<td>65 ± 8†</td>
<td>4.3 ± 0.4†</td>
</tr>
<tr>
<td>Burn (excised)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before endotoxin</td>
<td>4</td>
<td>48 ± 6</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>After endotoxin (6 hr)</td>
<td>4</td>
<td>71 ± 12†</td>
<td>3.8 ± 0.6</td>
</tr>
</tbody>
</table>

*Described previously.6,8
†Significantly different from control, p < 0.05.
Statistical analysis. Within each group, paired data were analyzed with Dunnett's t test comparing the individual time periods with baseline, whereas between-group unpaired data were compared at the individual time periods with Student's unpaired t test. Data between groups were compared with the Wilcoxon sign rank test; p < 0.05 was considered significant.

RESULTS

Group mean data are shown in the Tables I and II and Figs. 1 through 3. All 10 sheep survived the study period. The sheep that had undergone excision and grafting appeared to remain comfortable throughout the 3-day postexcision period. There was no drainage or erythema noted around the grafted site, and the grafted hide appeared to be well adhered to the underlying wound.

After burn, before endotoxin. There were no physiologic differences between the parameters obtained before burn injury and 3 days after burn and excision. Arterial and venous plasma conjugated dienes were also unchanged. This response was significantly different from that of the burned and nonexcised sheep, which had a 25% to 30% increase in oxygen consumption (\( \text{VO}_2 \)) during the 3-day period. In addition, the nonexcised sheep were noted to have a significant increase in venous conjugated dienes. In the four sheep killed at 4 days, we noted no increase in lung MDA or lung inflammation. Both inflammation and increased MDA were evident in the animals with a nonexcised burn at 3 days.

Postendotoxin physiologic response. A typical two-phase pulmonary response as seen with endotoxin alone was noted in sheep with burn excision. An initial increase in pulmonary artery pressure and lung lymph flow and decrease in arterial oxygen tension were present. However, the degree of initial hypertension and hypoxia was significantly less than that seen with endotoxin alone. The increased permeability phase 3 to 5 hours after endotoxin was characterized by a threefold to fourfold increase in protein-rich lung \( Q_L \). This response was not significantly different from that of endotoxin alone. However, the \( Q_L \) response was significantly less than the response seen in the group with nonexcised burns treated with endotoxin.

The systemic response was characterized by an initial decrease in cardiac output and increase in oxygen extraction from hemoglobin such that \( \text{VO}_2 \) was not dif-
Fig. 3. The lipid peroxidation response of sheep with excised burns (O) given endotoxin as measured by circulating conjugated (CONJ) dienes is compared with that of intact burned (●) and nonburned (□) sheep. There were no differences noted in conjugated diene levels between preburn and 3-day postburn values in the group with excised burns. Arterial conjugated dienes increased in the group with excised burns compared with venous conjugated dienes. The increase was significantly less than that seen with endotoxin alone and significantly greater than that seen with endotoxin given to the group with intact burns. Venous conjugated dienes did not increase in the excised or the nonburned sheep given endotoxin. Levels in the intact burned sheep were significantly elevated even before endotoxin, indicating ongoing systemic lipid peroxidation.

DISCUSSION

Generalized tissue inflammation has now been recognized to occur in the very early period after soft-tissue trauma, in particular burns. The initial response has been hypothesized to be at least in part responsible for the later onset of sepsis syndrome with multisystem organ failure frequently seen in the posttrauma period even in the absence of any documented infection. The initial inflammatory process has been reported to be caused by an initial oxygen radical release after burn injury that leads to systemic complement activation. A generalized lipid peroxidation process then occurs as a result of the early oxidant release. The role of this lipid peroxidation in the actual disease process remains speculative.
We have found that the early tissue inflammation can lead to an accentuated response to a later septic insult. The tissue inflammatory cells, which we have demonstrated to persist for at least 3 days after burn injury, can be primed by the initial stimulus, resulting in an accentuated release of oxidants, proteases, etc., by a second stimulus. In addition, cell membrane lipid peroxidation has been reported to increase in vitro to increase the potential for a subsequent oxidant injury.

We have also demonstrated that the postburn lipid peroxidation process also continues in the postresuscitation period, as evidenced by increased circulating conjugated dienes and lung tissue MDA content, both monitors of lipid peroxidation. The continuing oxidant changes could accentuate the septic or endotoxin tissue damage, also caused by oxidants, although other mediators released from ongoing inflammation could be causative.

We have recently found that endotoxin given 3 days after a 15% total body surface uninfected burn results in a marked initial increase in VO₂ followed by a decrease in VO₂ below normal with an increasing mixed venous oxygen saturation, suggestive of blood flow maldistribution. In addition, an increase in systemic soft-tissue permeability was noted.

The source of the mediators that amplify particularly the systemic septic response to endotoxin after burn injury is of considerable importance. Acute lung inflammation in the absence of infection has been clearly demonstrated to result in a sepsis syndrome leading to both increased tissue oxygen demands and impaired oxygen extraction. On the other hand, the burn wound is also an obvious source of inflammation. We have reported that complete excision and closure of the wound prevents the increased VO₂ in the postburn period in the uninfected sheep, indicating the causal relationship between burn inflammation and hypermetabolism. However, previous studies have not determined if the presence of an uninfected burn is also responsible for the accentuated response to endotoxin.

In this study we noted that the presence of the burn wound was in fact responsible for several aspects of the postburn process. First, the postburn increase in circulating lipid peroxides was prevented by early excision, as was the continued lung lipid peroxidation, indicating that these processes were being perpetuated by the presence of the wound. Second, we noted that the burn-induced lung inflammatory response, which was still present 3 days after burn injury, with the wound present, was considerably less in the sheep that had undergone excision during this time period. Third, we noted that the systemic response to endotoxin after burn injury was not accentuated if the burn was initially excised. This latter finding does not necessarily indicate that the source of the mediators responsible for the accentuated endotoxin response come directly from the burn wound, because the presence of the wound also appears to perpetuate the lung inflammation and lung lipid peroxidation. The burn could act as a continued stimulus to complement activation. The burn wound is also a source of endotoxin, although an endotoxin tolerance may also result with continued endotoxemia decreasing a subsequent systemic response to endotoxemia. However, early wound excision has also been reported to decrease circulating endotoxin in burned patients.

Of interest was the finding of a less severe early pulmonary hypertension and hypoxia and also lung lipid peroxide release from the burn-excised lung compared with endotoxin alone. The initial pulmonary hypertension is believed to be the result of an early massive thromboxane release that may in fact be oxidant induced, because antioxidant catalase infusion attenuates the process. The subsequent release of lipid peroxides measured as increased arterial conjugated dienes was also decreased. A lower value of lung MDA was also seen in the endotoxin injury with an intact burn. We hypothesize that the mechanism for this response was not a decrease in oxidant release with the burn but rather an increase in the clearance of products of lipid peroxidation through antioxidant activity. It is well recognized that a prior oxidant insult can increase endogenous lung antioxidant activity. Increased tissue glutathione peroxidase is considered to be responsible for the detoxification and clearance of lipid peroxides. The lack of correlation of lipid peroxidation and lung injury could also be because oxidants are not the major causative agent, since a cause-and-effect relationship between oxidants and tissue injury remains controversial.

We can conclude that early excision of the burn wound prevents the accentuated system response to a subsequent endotoxin challenge in the postburn resuscitation period. Early removal and closure of the burn wound also decrease the degree of lipid peroxidation and lung inflammation seen in the 3-day postburn period, as well as prevent postburn hypermetabolism.

REFERENCES
5. Demling RH, LaLonde C. Early postburn lipid peroxidation: