Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep

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1Department of Plastic and Reconstructive Surgery, Tokyo Women's Medical College, Tokyo, Japan; and Departments of 2Pediatrics, 3Anesthesiology and Physiology and Biophysics, and 4Pathology, The University of Texas Medical Branch, and Shriners Burns Institute, Galveston Texas 77555-0823

Sakurai, Hiroyuki, Frank C. Schmalstieg, Lillian D. Traber, Hal K. Hawkins, and Daniel L. Traber. Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep. J. Appl. Physiol. 86(4): 1151-1159, 1999.—The effects of a monoclonal antibody against L-selectin [leukocyte adhesion molecule (LAM)1–3] on microvascular fluid flux were determined in conscious sheep subjected to a combined injury of 40% third-degree burn and smoke inhalation. This combined injury induced a rapid increase in systemic prefemoral lymph flow (sQ lymph) from the burned area and a delayed-onset increase in lung lymph flow. The initial increase in sQ lymph was associated with an elevation of the lymph-to-plasma oncotic pressure ratio; consequently, it leads to a predominant increase in the systemic soft tissue permeability index (sPI). In an untreated control group, the increased sPI was sustained beyond 24 h after injury. Pretreatment with LAM1–3 resulted in earlier recovery from the increased sPI, although the initial responses in sQ lymph and sPI were identical to those in the nontreatment group. The delayed-onset lung permeability changes were significantly attenuated by pretreatment with LAM1–3. These findings indicate that both leukocyte-dependent and -independent mechanisms are involved in the pathogenesis that occurs after combined injury with burn and smoke inhalation.

systemic prefemoral lymph; lung lymph; fluid balance; hemodynamics; sheep

THERMAL INJURY GREATLY ALTERS microvascular fluid flux at the injury site. In patients with a large body surface area burn, this “capillary leak” leads to an intravascular volume depletion, rapidly requiring a large amount of fluid for resuscitation. Additional smoke inhalation induces further hemodynamic instability and increases fluid requirements (9, 35, 45). It is well known that both thermal injury and smoke inhalation involve inflammatory processes, although the details of pathogenesis and the time course for development of physiological manifestations are different (44).

Leukocytes, particularly polymorphonuclear neutrophils (PMNs), are central mediators of inflammatory processes and play a significant role in the pathogenesis of both thermal injury (29, 34) and smoke inhalation (5). Endothelial injury by activated PMNs is thought to occur as a result of the release of proteases and toxic oxygen products, leading to increases in microvascular permeability and edema formation. The selectin family of adhesion-promoting molecules, including L-, E-, and P-selectins, appears to be involved in the earliest events of the acute inflammatory processes. The initial adhesive interactions between PMNs and endothelial cells (ECs) by the selectin family result in the “rolling” phenomenon, whereby PMNs assume an intermittent adhesive contact with ECs (2, 11). The second phase for the firm adhesive interaction appears to depend on engagement of the β2-integrins (CD11/CD18) on the PMNs and the intracellular adhesion molecule (ICAM)-1 or ICAM-2 on the ECs (42).

Because the rolling phenomenon is the first step of adhesion, it seems reasonable to consider an immunoneutralization of the selectin family as a potentially therapeutic intervention for patients with severe thermal injury. Accumulating experimental evidence suggests that the inhibition of selectins reduces PMNs' accumulation into the tissues and permeability changes, not only in the local burned skin but also in distant organs, including the lung (19, 34, 38). Within this paradigm, one might expect that reduced endothelial injury by inhibition of PMN-EC adherence might cause significantly less fluid loss and consequently an early establishment of hemodynamic stability after severe thermal injury. However, only a few studies (30) have examined physiological manifestations to determine the effect of inhibition of the PMN-EC interactions.

Accordingly, the present study was undertaken to test the effect of an anti-L-selectin antibody [leukocyte adhesion molecule (LAM)1–3] on physiological manifestations after severe thermal injury. Of particular interest were alterations in the microvascular fluid flux in both burned tissue and lung. L-selectin was chosen as a therapeutic approach, as selectins located on the ECs (P- and E-selectin) exhibit organ-specific differences in their pathophysiological relevance (19, 33). We used a combined-injury model with 40% body surface area (BSA), third-degree burn, and 48 breaths of cotton smoke inhalation to mimic the hemodynamic alterations often seen in patients with severe burn injury.

MATERIALS AND METHODS

Animals were cared for in the Ovine Intensive Care Unit at our institution (Univ. of Texas Medical Branch), which is approved by the American Association of Laboratory Animal Care. The experimental procedures were approved by the Animal Care and Use Committee of The University of Texas Medical Branch. The National Institutes of Health and...
American Physiological Society guidelines for animal care were strictly followed. Animals were studied in the awake state.

Antibody development and testing. Purified LAM1–3 (47), a murine monofunctional anti-human L-selectin antibody that interferes with PMN attachment to human umbilical vein ECs, was obtained from Xenotech (San Mateo, CA). Each lot of antibody was tested by the Limulus assay (24) and was free of endotoxin.

Before this study, we performed in vitro studies to ensure the ability of this antibody to block adherence of ovine neutrophils to ECs. Ovine neutrophil attachment to ECs was examined, as previously described for human neutrophils (27), with the following modifications. Briefly, sheep venous ECs were grown to confluence in eight-well tissue culture Lab-Tek chambers or on slides (Miles, Naperville, IL). The cells were stimulated with interleukin (IL)-1 culture (105) at 4°C were added to the wells with or without LAM1–3 (100 µg/ml) in a final volume of 200 µl. The cells were allowed to adhere under static conditions for 15 min. At the end of this time, the adherent cells were placed on a rotating platform at ~75 rpm for 15 min. The wells were gently washed twice with buffer, and the cells were fixed in paraformaldehyde and counted. The number of neutrophils adhering to the stimulated sheep endothelium with LAM1–3 (normalized to 100% binding for cells without antibody) was 31 ± 6 (SE)%. These values for LAM1–3 are similar to that found for human ECs and neutrophils (27), ensuring the functional cross-reaction of LAM1–3 to the ovine neutrophils.

Surgical preparation. Twenty-one female range-bred adult sheep (32–42 kg) were surgically prepared for study. All animals were intubated via an endotracheal tube and ventilated during the surgery while under halothane anesthesia. Arterial and venous catheters (16 G, 24 in., Intraclip, Becton-Dickinson, Sandy, UT) were placed in the descending aorta and inferior vena cava via the femoral artery and vein, respectively. A Swan-Ganz thermal dilution catheter (model 690C, Siemens-Elema, Solna, Sweden) was inserted in the pulmonary artery via the right external jugular vein. The chest was opened at the fifth intercostal space on both sides, and an effluent lymphatic from the caudal mediastinal lymph node was cannulated (Silastic medical-grade tubing, 0.025 in. ID, 0.047 in. OD, Dow Corning, Midland, MI) by a modification of the technique of Staub et al. (48). The systemic contribution was removed by ligation of the tail of the caudal mediastinal lymph node and cauterization of the systemic diaphragmatic lymph vessels. A Silastic catheter was also positioned in the left atrium to measure left atrial pressure directly. An incision was made anterior to the right tensor fascia lata, and an effluent lymphatic from the systemic prefemoral lymph node was cannulated by using the method of Demling et al. (10). The sheep were given 5–7 days to recover from the surgical procedure with free access to food and water.

Burn and smoke inhalation injury. Before the injury was produced, all animals received a tracheostomy and auffed tracheostomy tube (10-mm diameter, Shiley, Irvine, CA) was inserted by using 10 mg/kg of ketamine (Ketalar, Parke-Davis, Morris Plains, NJ). Then, the anesthesia was continued with 2–3% halothane and 50% oxygen. Sixteen sheep then received a combined injury with a 40% third-degree burn and 48 breaths of cotton smoke inhalation. After the wool over the bilateral flank was shorn, a 20% total body surface third-degree flame burn was given to the flank of one side. The BSA was calculated from the equation BSA = 0.084 × body wt (kg)2/3 (12). Burn was produced with a Bunsen burner, until the skin was thoroughly contracted. We have previously determined this degree of injury to be a full-thickness burn, i.e., including both epidermis and dermis, in which the nerve endings are heat destroyed (7). Thereafter, inhalation injury was induced while the sheep was in the prone position. A modified bee smoker was filled with 50 g of burning toweling and was connected to the tracheostomy tube. The connection contained a thermistor to monitor the temperature of the smoke. During the insufflation procedure, the temperature of the smoke did not exceed 40°C. The sheep were insufflated with 48 breaths (650 ml/breath) of cotton smoke. The details of the smoking procedure and the chemical composition of the smoke have been previously described (26). After smoke insufflation, another 20% BSA third-degree burn was given to the flank of the other side. A Foley catheter was placed in the bladder to determine urine output. During this procedure, ~30 min of anesthesia were required.

Experimental protocol. On the day of the injury, baseline measurements were obtained. Two hours before injury, seven sheep (LAM1–3 group) received LAM1–3 (1.0 mg/kg body wt dissolved in 10 ml 0.9% NaCl) as a bolus intravenous injection. Adequate concentrations of LAM1–3 at 48 and 72 h were documented by using flow cytometry to demonstrate that the L-selectin on normal neutrophils (1 × 106 cells/ml) placed in plasma from these animals was completely saturated. Nine animals (nontreatment group) did not receive this treatment. All 16 animals were given the combined injury with 40% third-degree burn and 48 breaths of cotton smoke described in the previous subsection. Five animals (control group) underwent the same procedure, including the tracheostomy and anesthesia, but did not receive any injury. Immediately after these procedures, anesthesia was discontinued and physiological measurements were serially determined at 3, 6, 12, 18, 24, 36, 48, and 72 h after insult, while all animals were resuscitated after the protocol described in the following subsection.

Resuscitation protocol. Immediately after injury, anesthesia was discontinued and the animals were allowed to awaken but were mechanically ventilated with a Servo Ventilator 900C (Siemens-Elema, Solna, Sweden); Swensa thereafter, the setting was adjusted to maintain the arterial oxygen saturation above 90%. These respiratory settings allowed a rapid disappearance of carboxyhemoglobin after smoke inhalation (26).

Fluid resuscitation during the experiment was performed with Ringer lactate solution following the Parkland formula (6) (4 ml %burned surface area ·kg body wt −1 for the first 24 h and 2 ml %burned surface area ·kg body wt −1 ·day −1 for the next 48 h). One-half of the volume for the first day was infused in the initial 8 h, and the remainder was infused in the next 16 h. Urine was collected, and urine output was recorded every 24 h. Fluid balance was determined by urine output every 24 h subtracted from total fluid volume infused and was represented as ml ·kg −1 ·day −1. During this experimental period, the animals were allowed free access to food, but not to water, for determination of accurate fluid balance.

Because the resuscitation protocol itself might affect the physiological parameters, even the animals in the control group received the identical amount (ml/kg body wt) of fluid resuscitation and underwent the same ventilatory support as...
injured animals during the whole experimental period. When all measurements were completed, all animals were anesthetized with ketamine and humanely killed by administration of a saturated potassium chloride solution.

Hemodynamic and oxygenation variables. Measured physiological parameters were not considered valid until the animals were fully awake and standing. Hemodynamic and oxygenation variables were measured and calculated according to standard formulas. Cardiac output was measured with a cardiac output computer (model 9520, American Edwards) by the thermodilution method with 5% dextrose as an indicator solution, then divided by BSA to determine the cardiac index (CI). Vascular pressures were measured by using fluid-filled pressure transducers (P23ID, Statham Gould, Oxnard, CA) adapted to a continuous flushing device and connected to a physiological recorder (model OM9 patient monitor, Electronics for Medicine, Honeywell, Pleasantville, NY). Zero calibrations were taken at the level of the olecranon joint on the front leg, which is considered to be the level of the right atrium. At every time point, arterial and mixed venous blood were determined (models 1302 pH/blood-gas analyzer and 282 CO-oximeter, Instrumentation Laboratory, Lexington, MA). The blood-gas results were corrected for the body temperature of the sheep.

Lymph and plasma measurements. Systemic prefemoral lymph flow (sQ lymph) and lung lymph flow (Q lymph) were measured with a graduated test tube and stopwatch. Lymph and blood samples were collected in EDTA tubes, and then the colloid osmotic pressure in plasma (πp), systemic prefemoral lymph (πp,L) were determined through a semi-permeable membrane in a colloid osmometer (model 4100, Wescor, Logan, UT). Systemic permeability index (sPI) and lung permeability index (PL,L) were calculated according to the following equations

\[
sPI = \frac{sQ_{\text{lymph}}}{s\pi_p}\times \frac{s\pi_p}{s\pi_p}
\]

\[
PL,L = \frac{Q_{\text{lymph}}}{\pi_p,L}\times \frac{\pi_p,L}{s\pi_p}
\]

Analysis of data. All values are reported as means ± SE. Outcome variables for physiological parameters were analyzed by using analysis of variance for a two-factor experiment with repeated measures on time. The two factors are experimental groups (control, nontreatment, and LAM1–3 group) and time (9 time points, including baseline). Fluid balance data for each day were analyzed by using one-way analysis of variance. Fisher’s least-significant-difference procedure was used for multiple comparisons, with Bonferroni correction for number of comparisons. All tests were assessed at the 0.05 level of significance.

RESULTS

Two of nine animals in the nontreatment group and one of seven animals in the LAM1–3 group did not survive the entire experimental period. The survival time after injury of these three animals was 48, 60, and 68 h, respectively. The CI was 7.80, 8.24, and 7.04 l·min⁻¹·m⁻², respectively, immediately before death. On the other hand, the arterial Po₂-to-inspiratory oxygen fraction ratio (P/F) was 59, 52, and 65 Torr, respectively, indicating that the major cause of death was due to cardiopulmonary dysfunction by inadequate fluid resuscitation but to progressive deterioration in oxygenation. The data from these three animals were discarded from further analysis. All other animals survived the 72-h experimental period, with normal appetite and intake of food.

All animals received an identical amount of fluid following the Parkland formula, which was applied to the 40% BSA thermal injury. Without any injury, the control group exhibited an intake-dependent urine output (Fig. 1). The animals that received the combined injury (the nontreatment group and the LAM1–3 group) showed significantly less urine output than did the control group in the first and second days after injury. During the first day, urine output in the LAM1–3 group was significantly higher than in the nontreatment group.

The summarized cardiopulmonary hemodynamic data are shown in Table 1. After the combined injury with burn and smoke inhalation, there was a transient decrease in CI, whereas the control group did not show any significant alterations during the whole experimental period. The decreased CI in the nontreatment group and LAM1–3 group gradually returned toward baseline values, and at 72 h after injury it was increased from the baseline value. Despite the initial decrease in CI, mean arterial pressure was maintained in both nontreatment and LAM1–3 groups. Pulmonary arterial pressure was increased in all groups under mechanical ventilation with positive end-expiratory pressure. There was no statistically significant difference between the nontreatment and the LAM1–3 groups in hemodynamic variables at any time.

Table 2 depicts the oxygenation data in both groups. The arterial blood Po₂ was well maintained in all groups with ventilatory support. Progressively deteriorating oxygenation in the injured animals was represented as a significant decrease in the P/F 12 h after the combined injury with burn injury and smoke inhalation. There was no statistical difference between the nontreatment group and LAM1–3 group in these oxygenation parameters.

\[
\pi_p, s\pi_p, \text{ and } \pi_p,L \text{ are shown in Table 3. After combined injury, } \pi_p \text{ rapidly decreased in the nontreatment and } \text{the LAM1–3 groups. Lymph oncotic pressures also}
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\[
\text{Fig. 1. Urine output in all 3 groups for each day. All animals received an identical amount of fluid}
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\[
\text{following the Parkland formula, which was applied to 40% body surface area (BSA) thermal injury. Open}
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\[
\text{bars, control group; hatched bars, untreated group; solid bars, treatment group. †Significantly different from}
\]

\[
\text{control group, P < 0.05. †Significantly different from nontreatment group, P < 0.05.}
\]
from baseline value. †Significant difference (P < 0.05) vs. control group.

Table 1. Cardiopulmonary hemodynamics

<table>
<thead>
<tr>
<th>Variable/Group</th>
<th>Baseline</th>
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<th>24</th>
<th>48</th>
<th>72</th>
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<tr>
<td>CI (l·min⁻¹·m⁻²)</td>
<td>5.60 ± 0.37</td>
<td>5.46 ± 0.26</td>
<td>5.66 ± 0.26</td>
<td>5.97 ± 0.25</td>
<td>6.01 ± 0.37</td>
<td>5.59 ± 0.38</td>
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<tr>
<td>Non-treatment</td>
<td>5.77 ± 0.29</td>
<td>4.55 ± 0.15†</td>
<td>4.67 ± 0.10†</td>
<td>5.26 ± 0.46</td>
<td>6.48 ± 0.32</td>
<td>6.99 ± 0.25†</td>
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<td>LAM1–3</td>
<td>5.52 ± 0.15</td>
<td>4.74 ± 0.19*</td>
<td>4.79 ± 0.17†</td>
<td>5.48 ± 0.31</td>
<td>5.97 ± 0.35</td>
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<td>MAP, mmHg</td>
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<td>100 ± 3</td>
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<td>105 ± 5</td>
<td>104 ± 4</td>
<td>106 ± 6</td>
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<td>105 ± 5</td>
<td>104 ± 4</td>
<td>106 ± 7</td>
<td>116 ± 6</td>
<td>115 ± 6*</td>
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<td>LAM1–3</td>
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<td>97 ± 7</td>
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<td>PAP, mmHg</td>
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<td>24 ± 1*</td>
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<td>26 ± 1*</td>
<td>28 ± 1†</td>
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<td>30 ± 1†</td>
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<td>9 ± 1</td>
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<td>10 ± 1</td>
<td>12 ± 1*</td>
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<td>10 ± 1*</td>
<td>11 ± 1*</td>
<td>12 ± 1*</td>
<td>11 ± 1*</td>
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Values are means ± SE. CI, cardiac index; MAP, mean arterial pressure; PAP, pulmonary arterial pressure; CVP, central venous pressure; LAP, left atrial pressure; LAM1–3, leukocyte adhesion molecule, monoclonal antibody against L-selection. * Significant difference (P < 0.05) from baseline value. † Significant difference (P < 0.05) vs. control group.

Table 2. Oxygenation data

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<td>FlO₂</td>
<td>0.21</td>
<td>1.00</td>
<td>0.21 ± 0.00</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.21 ± 0.00</td>
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</tr>
<tr>
<td>Non-treatment</td>
<td>0.21</td>
<td>1.00</td>
<td>0.55 ± 0.06</td>
<td>0.42 ± 0.04</td>
<td>0.59 ± 0.09</td>
<td>0.63 ± 0.09</td>
<td>0.74 ± 0.09</td>
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<tr>
<td>LAM1–3</td>
<td>0.21</td>
<td>1.00</td>
<td>0.43 ± 0.04</td>
<td>0.36 ± 0.04</td>
<td>0.63 ± 0.12</td>
<td>0.64 ± 0.09</td>
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<td>PAO₂, Torr</td>
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<td>Control</td>
<td>99 ± 6</td>
<td>473 ± 21*</td>
<td>112 ± 12</td>
<td>127 ± 13*</td>
<td>123 ± 16*</td>
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<td>Non-treatment</td>
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<td>P/F ratio</td>
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<td>Control</td>
<td>472 ± 30</td>
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<td>542 ± 20</td>
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<td>391 ± 52†</td>
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<td>200 ± 35†</td>
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<tr>
<td>LAM1–3</td>
<td>469 ± 17</td>
<td>479 ± 22</td>
<td>482 ± 22</td>
<td>398 ± 50†</td>
<td>226 ± 68†</td>
<td>216 ± 34†</td>
<td>196 ± 60†</td>
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Values are means ± SE. FlO₂, inspired O₂ fraction; PAO₂, partial pressure of O₂ in arterial blood; P/F ratio, PAO₂-to-FIO₂ ratio. * Significant difference (P < 0.05) from baseline value. † Significant difference (P < 0.05) vs. control group.

Table 3. Plasma and lymph colloid osmotic pressure

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<th>Variable/Group</th>
<th>Baseline</th>
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<th>12</th>
<th>24</th>
<th>48</th>
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<td>π₀, mmHg</td>
<td>16.8 ± 0.7</td>
<td>16.2 ± 1.0</td>
<td>16.1 ± 1.0</td>
<td>16.5 ± 0.7</td>
<td>17.0 ± 0.8</td>
<td>16.9 ± 0.8</td>
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<td>Control</td>
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<tr>
<td>Non-treatment</td>
<td>16.5 ± 0.8</td>
<td>11.6 ± 0.4†</td>
<td>10.9 ± 0.5†</td>
<td>9.3 ± 0.2†</td>
<td>9.8 ± 0.5†</td>
<td>10.5 ± 0.3†</td>
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<tr>
<td>LAM1–3</td>
<td>18.5 ± 1.3</td>
<td>13.5 ± 0.9†</td>
<td>13.4 ± 1.1*</td>
<td>12.5 ± 1.2†</td>
<td>12.0 ± 1.3†</td>
<td>12.1 ± 1.2†</td>
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<td>sπ₀, mmHg</td>
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<tr>
<td>Control</td>
<td>8.6 ± 0.2</td>
<td>6.1 ± 1.0*</td>
<td>9.3 ± 1.1</td>
<td>8.2 ± 0.5</td>
<td>9.3 ± 0.8</td>
<td>8.8 ± 0.9</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>8.9 ± 0.8</td>
<td>8.4 ± 0.6</td>
<td>7.3 ± 0.3</td>
<td>6.0 ± 0.3†</td>
<td>4.7 ± 0.5†</td>
<td>3.8 ± 0.7†</td>
</tr>
<tr>
<td>LAM1–3</td>
<td>10.1 ± 1.7</td>
<td>9.0 ± 0.9</td>
<td>8.0 ± 0.6</td>
<td>6.3 ± 0.6*</td>
<td>5.0 ± 0.7†</td>
<td>4.3 ± 0.6†</td>
</tr>
<tr>
<td>π₁, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.4 ± 0.7</td>
<td>9.3 ± 0.9</td>
<td>9.3 ± 0.6</td>
<td>9.0 ± 0.5</td>
<td>9.7 ± 0.9</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>9.6 ± 1.1</td>
<td>6.7 ± 0.7</td>
<td>6.3 ± 0.7†</td>
<td>5.4 ± 0.6†</td>
<td>5.5 ± 0.7†</td>
<td>5.1 ± 0.5†</td>
</tr>
<tr>
<td>LAM1–3</td>
<td>11.8 ± 1.7</td>
<td>9.0 ± 0.9</td>
<td>8.0 ± 0.6*</td>
<td>6.3 ± 0.6*</td>
<td>5.0 ± 0.7†</td>
<td>4.3 ± 0.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. π₀, Plasma colloid osmotic pressure; sπ₀, systemic prefemoral lymph colloid osmotic pressure; π₁, lung lymph colloid osmotic pressure. * Significant difference (P < 0.05) vs. baseline value. † Significant difference (P < 0.05) vs. control group.
gradually decreased in both injured groups. However, during the initial 12 h the decrease in $s\pi_t$ was not significantly different from the baseline values of these two groups.

Systemic soft tissue (burned tissue in the nontreatment and the LAM1–3 groups) microvascular fluid flux is shown in Fig. 2. After injury, there was a rapid increase in $sQ_{\text{lymph}}$, in both nontreatment and LAM1–3 groups. This increase was accompanied by a transient increase in lymph-to-plasma oncotic pressure ratios; consequently, $s\pi$ was significantly increased immediately after injury. In the control group, there was a mild but significant increase in the $sQ_{\text{lymph}}$, with a large amount of fluid infusion. However, this increase was associated with a transient decrease in the $s\pi_t/s\pi_p$; therefore, in the control group $s\pi$ was essentially unchanged during the whole experimental period. Increased $s\pi$ in both the nontreatment group and the LAM1–3 group subsided 72 h after injury. The peak of the increased $s\pi$, however, seems to be different between the two groups; in the nontreatment group, increased $s\pi$ was sustained more than 24 h after injury, whereas that in the LAM1–3 group began to subside earlier, creating a significant difference at 48 h after injury.

Figure 3 depicts the pulmonary transvascular fluid flux. In the nontreatment group, a significant increase in $Q_{\text{Lymph}}$ was noted 12 h after injury. This increase was significantly attenuated by pretreatment with LAM1–3. In contrast to the burned tissue lymph, $s\pi_t/s\pi_p$ was virtually unchanged during the whole experimental period in all groups. Consequently, increased $PI_{L}$ after the combined injury was significantly attenuated by the pretreatment with LAM1–3 at 24 h after injury.

**DISCUSSION**

Because we wished to determine the role of L-selectin on physiological manifestations in the severe thermal injury model, reproducible hemodynamic responses to injury are essential in this study. It is well known that hemodynamic responses to thermal injury in the acute phase and those in the postresuscitation phase are totally different (53) and are significantly modified by fluid resuscitation regimens (32). In the present study, the Parkland formula for fluid resuscitation, which is most widely accepted among multiple institutions (13), was strictly followed during the whole experimental period. Animals that were subjected to the combined injury with 40% third-degree burn and severe smoke inhalation exhibited a biphasic hemodynamic response. The initial hemodynamic change was characterized as a decrease in $CI$, whereas the mean arterial pressure was easily maintained by fluid resuscitation. Thereafter, $CI$ gradually increased toward baseline value and exceeded it at 72 h after injury. These findings are commonly observed in patients with severe thermal injury (53).

Most of these hemodynamic alterations might be attributable to increased microvascular fluid flux in the
burned tissue and subsequent fluid shift from the vascular space to the interstitial space. This capillary leak was manifested as a rapid increase in $sQ_{\text{lymph}}$, $sp_i/p_p$, and $sPI$. Pretreatment with LAM1–3 did not show any effect on these alterations, suggesting that microvascular changes immediately after thermal injury were not L-selectin dependent. A number of mediators, such as histamine (17), bradykinin (41), serotonin (15), prostaglandins (4), and leukotrienes (3), may contribute to rapid permeability changes after thermal injury. Toxic oxygen products also play a major role in local permeability changes after thermal injury, because antioxidant enzymes, such as catalase or superoxide dismutase, attenuate early edema formation in thermally injured animals (51). The source of these reactive oxygen intermediates remains unclear. Not only microvascular permeability but also physical alterations in the interstitial space contribute to the rapid increase in fluid flux. Lund et al. (28) demonstrated strongly negative hydrostatic pressure in the dermal interstitium immediately after thermal injury. Data from the present study are compatible with previous observations indicating that initial burn edema formation does not require the presence of PMNs.

Despite an inability to attenuate the initial transvascular fluid flux in the burn wound, the anti-L-selectin antibody seems to have an effect on burned tissue permeability in the later period after burn. The increased $sPI$ in the nontreatment group was sustained beyond 24 h after injury, whereas that in the LAM1–3 group began to subside before 24 h. Mulligan et al. (34) used a second-degree thermal injury model in rats and reported that neutrophil depletion reduced edema formation 4 h after thermal injury but did not reduce it 1 h after injury. In their report (34), a monoclonal antibody against L-selectin also attenuated edema formation in burned skin 4 h after injury. The findings in the present study are in accordance with their reports (34), suggesting that neutrophil-dependent edema formation does not occur immediately but in the later period after thermal injury. A large discrepancy in the time scale for developing neutrophil dependency [i.e., 4 h after injury in the study by Mulligan et al. vs. more than 24 h after injury in the present study] might be the result of different injury models between the two studies. Rapid hyperemic changes are consistent findings in second-degree burn wounds (16, 39). On the other hand, large third-degree burns induce significant decreases in blood flow to the burn wound (14). Systemic hemodynamic alterations associated with larger burns may accentuate this low-perfusion state in the burned tissue. An initially decreased vascular bed available for adherence

![Fig. 3. Changes in pulmonary microvascular fluid flux in 3 groups over time. A: lung lymph flow ($Q_{\text{lymph}}$). B: $sp_i/p_p$. C: lung permeability index ($PI_{L}$). *Significantly different from baseline values, $P < 0.05$. †Significantly different from control group, $P < 0.05$. # Significantly different from nontreatment group, $P < 0.05$.](image-url)
in third-degree burn might be related to the later involvement of neutrophils in the present study. With respect to the pathogenesis inducing permeability changes, there appears to be a distinct difference between thermal injury and smoke inhalation. In contrast to the immediate alterations in burn wound permeability, lung edema associated with smoke inhalation is generally delayed in onset (23, 40, 44). This distinct difference was consistently reproduced in the present combined-injury model. In the nontreatment group, a significant increase in Q˙Llymph was observed in PI,L was also noted more than 6 h after injury. Therefore, a significant increase in PI,L was also noted more than 6 h after injury.

Toxic oxygen products are involved in these delayed-onset pulmonary microvascular derangements (25, 36). We have previously shown that depletion of the PMNs from sheep virtually eliminated the pulmonary permeability changes seen after smoke inhalation (5). On the other hand, inhibition of xanthine oxidase (XO), another source of oxygen radicals, does not attenuate lung fluid flux after smoke inhalation (1), whereas XO inhibitors are reported to reduce rapid edema formation in burn wounds (51). These observations suggest the source of oxygen free radicals after smoke inhalation may be different from that in the acute burn wound. In the present study, immunoneutralization of L-selectin significantly attenuated the increase in pulmonary vascular permeability. This finding supports previous observations indicating that PMNs play a central role in delayed-onset pulmonary microvascular derangements after smoke inhalation.

Although LAM1–3 reduced the delayed-onset pulmonary microvascular permeability changes, it did not attenuate the oxygenation deficit in this combined-injury model. The mechanisms by which the progressive hypoxia occurred after smoke inhalation are complicated and are not fully explained by the alveolar phenomenon secondary to increased pulmonary microvascular permeability. In the present study, recovering pulmonary microvascular permeability 48–72 h after injury did not induce improvement in oxygenation in either nontreated or treated groups. Bronchial phenomena, such as bronchospasm, peribronchial constriction, or partial bronchial obstruction by cast formation, also contribute to the progressive hypoxia (20, 52). A recent investigation using the multiple-inert-gas elimination technique (46) demonstrated that pulmonary blood flow was recruited to the low ventilation-perfusion compartment at 24–72 h after smoke inhalation. Therefore, mechanisms other than increased pulmonary microvascular permeability seem to contribute to the progressive deterioration of oxygenation shown in the present study.

Pathological alterations in systemic microvascular phenotype remote from the lung have been described in various types of acute lung injury (8, 31, 49). There is increasing evidence indicating that systemic microvascular derangements with acute lung injury are mediated by activated neutrophils (18, 37, 43). St. John et al. (49) reported that increased intestinal microvascular permeability after acid aspiration can be prevented by inhibiting leukocyte adherence. In the present study, it is quite conceivable that sustained increases in sQ˙lymph and sPI in the nontreatment group are, at least in part, caused by activation of PMNs associated with smoke inhalation.

Although improved permeability change in the LAM1–3 group was manifested as less positive fluid balance during the first day, it did not result in an earlier establishment of hemodynamic stability. There was no significant difference in hemodynamic data between the nontreatment and LAM1–3 groups. Mileski et al. (30), using a 30% BSA third-degree burn model in rabbits, demonstrated that inhibition of leukocyte adherence reversed the hypotensive response, whereas cardiac output was maintained during the experimental period. However, interpretation of their findings relevant to the clinical settings is difficult, as decreased mean arterial pressure is not a typical hemodynamic response to thermal injury in humans (53). It is well documented that depressed cardiac myocontractility contributes to hemodynamic alterations after severe thermal injury (21, 50). Horton and White (22) reported that leukocyte depletion did not ablate burn-induced cardiac depression, whereas an XO inhibitor (allopurinol) or inactivation (tungsten-enriched diet) provided a measure of cardioprotection. Neutrophil-independent cardiac depression might lead to the discouraging effects of LAM1–3 on hemodynamics in the present study.

In summary, this combined-injury model satisfactorily reproduced the hemodynamic alterations often seen in severely burned patients. A distinct difference in pathogenesis was noted between thermal injury and smoke inhalation. Both neutrophil-dependent and independent mechanisms seem to be involved in this combined-injury model.

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L-SELECTIN IN BURN AND SMOKE INHALATION


