Effects of Recombinant Human Tumor Necrosis Factor Alpha, Lymphotoxin, and Escherichia coli Lipopolysaccharide on Hemodynamics, Lung Microvascular Permeability, and Eicosanoid Synthesis in Anesthetized Sheep

E.A. Kreil, E. Greene, C. Fitzgibbon, D.R. Robinson, and W.M. Zapol

We infused recombinant human tumor necrosis factor alpha (rhTNFα), lymphotoxin (rhLT), and Escherichia coli 0111:B4 lipopolysaccharide (LPS) into anesthetized sheep with a lung lymph fistula to compare their effects on systemic and pulmonary hemodynamics, lung lymph dynamics, and eicosanoid release. rhTNFα (25–150 μg/kg, n=6 sheep), but not rhLT (25 μg/kg, n=3), rapidly increased lung lymph and plasma levels of 6-keto-prostaglandin F1α (6-k-PGF1α) and caused profound systemic vasodilation and hypotension. Meclofenamate pretreatment (10 mg/kg) of three other sheep given 25 μg/kg rhTNFα prevented the increase of lymph and plasma 6-k-PGF1α levels, systemic vasodilation, and the early (<2 hrs) but not the late (4–6 hours) hypotension caused by rhTNFα. LPS (1 μg/kg, n=11) induced a briefer increase of lymph 6-k-PGF1α levels than did rhTNFα while plasma 6-k-PGF1α levels did not increase. LPS induced more gradual hypotension than did rhTNFα but did not cause systemic vasodilation. LPS and rhTNFα, but not rhLT, increased lymph thromboxane B2 (TXB2) levels during the first hour of study, whereas only LPS acutely increased plasma TXB2 levels. LPS caused acute pulmonary vasoconstriction and greater acute pulmonary artery hypertension than did either rhTNFα or rhLT. Whereas LPS-treated sheep required less fluid transfusion than rhTNFα-treated sheep to maintain mean systemic arterial pressure greater than 50 mm Hg, LPS infusion caused a greater increase of lung lymph protein clearance. rhTNFα caused minimal alterations of lung microvascular permeability. We conclude that eicosanoid mediators contribute importantly to differences of systemic and pulmonary hemodynamics caused by these agents in sheep. rhTNFα cannot account for all of the LPS-induced hemodynamic, lung lymph, and eicosanoid responses in sheep. (Circulation Research 1989;65:502–514)

Tumor necrosis factor alpha (TNFα) and lymphotoxin (LT, also named TNFβ) are structurally related cytokines that mediate or modulate inflammatory processes in response to a variety of foreign antigens.1–5 TNFα is produced by a number of cells, including macrophages/microcytes and natural killer cells, after activation by gram-negative bacteria, viruses, parasites, and lipopolysaccharide (LPS). TNFα can induce the release of other inflammatory mediators, including interleukin-6–8 and eicosanoids such as prostaglandin E2,9 and prostacyclin.10 Infusion of recombinant human TNFα (rhTNFα) into several animal species causes severe systemic hypotension, metabolic acidemia, multiple-organ damage, including hemorrhagic interstitial pneumonitis, and death within hours.11,12 Because these responses to TNFα infusion resemble the responses to LPS infusion, and because LPS causes increased serum levels of TNFα in both animals13 and humans,14 TNFα has been implicated as a major mediator of the lethal effects of LPS. Additional support for this hypothesis arises from studies in which pretreatment with...
Table of Abbreviations

Clt, lung lymph transvascular protein clearance
HR, heart rate
L/P, ratio of [lung lymph protein]/[arterial plasma protein]
LPS, lipopolysaccharide
LR, lactated Ringer's solution
M, meclofenamate
PAOP, pulmonary artery balloon occlusion pressure
PAP, mean pulmonary artery pressure
PVRI, pulmonary vascular resistance index
Qt, lung lymph flow
Qs/Qt, lung shunt fraction, as percent of right cardiac output
rhLT, recombinant human lymphotoxin
rhTNF, recombinant human tumor necrosis factor alpha
SAP, mean systemic arterial pressure
6-k-PGF, 6-keto-prostaglandin Fα
SVRI, systemic vascular resistance index
TXB₂, thromboxane B₂

anti-TNFα antibodies markedly improved survival rates of mice challenged with LPS
and baboons given infusions of live gram-negative bacteria.

In contrast, LT, which is produced by activated lymphocytes, was not detectable in serum by bioassay after LPS infusion into human volunteers. Although LT and TNFα share approximately 30% homology of amino acid sequence, appear to bind to a common receptor, and have many similar effects on endothelial cells, the role of LT in mammalian defense against infection is unknown. Furthermore, whereas LPS-induced lung injury has been studied extensively in animals, the roles of both TNFα and LT in the early evolution of LPS-induced lung injury are unknown.

Our study compared the effects of intravenous infusions of rhTNFα, rhLT, and Escherichia coli LPS on anesthetized sheep to discover qualitative differences of response to these agents. In addition to measuring pulmonary and systemic hemodynamics, we sought evidence of lung injury by measuring lung lymph flow, transvascular protein clearance, and lung extravascular water content. Levels of the eicosanoids thromboxane B₂ (TXB₂), the hydrolysis product of the pulmonary vasoconstrictor and bronchoconstrictor TXA₂, and 6-keto-prostaglandin F₁α (6-k-PGF₁α), a metabolite of the vasodilator prostacyclin, were measured by radioimmunoassay in plasma and lung lymph. Because we measured a marked increase of eicosanoid release after infusion of rhTNFα, we also studied the effects of a cyclooxygenase inhibitor in sheep given rhTNFα. To avoid hypovolemic shock caused by LPS or the cytokines, we attempted to maintain the mean systemic arterial pressure above 50 mm Hg and the pulmonary artery occlusion pressure (PAOP) at the baseline value ±2 mm Hg by transfusion of lactated Ringer's solution (LR).

Materials and Methods

Animal Preparation

Thirty-two Hampshire sheep (15–39 kg) were prepared for study. Each animal was anesthetized with 0.5–0.75% halothane (Halocarbon Laboratories, Hackensack, New Jersey), intubated, and ventilated by a constant-volume respirator (model 613, Harvard Apparatus, Millis, Massachusetts) with pure oxygen at a tidal volume of 11–12 ml/kg and a rate of approximately 12–14 breaths/min to establish the baseline (prestudy) arterial PCO₂ at 35–45 mm Hg. Positive end-expiratory pressure of 5 cm water was applied. Pancuronium chloride (Organon, West Orange, New Jersey), 0.5–1.0 mg, was infused every 2–4 hours to provide muscle relaxation.

Sterile femoral incisions were made, and polyvinyl catheters (North American Instrument, Hudson Falls, New York) were advanced to midthoracic level through both femoral arteries and one femoral vein to measure mean systemic arterial blood pressure (SAP) and heart rate (HR) and to sample blood. A 7.5F flow-directed thermocatheter pulmonary artery (PA) catheter (model 73R6057, Electracatheter, Rahway, New Jersey) was passed via an 8F introducer sheath with side-port (Cordis, Miami, Florida) through the right jugular vein to monitor mean pulmonary artery pressure (PAP) and PAOP.

An acute pulmonary lymph fistula was established in all sheep following the method of Staub et al. The efferent lymph duct of the caudal mediastinal node was cannulated with a silicone catheter (medical grade tubing, 0.64 mm i.d. x 1.19 mm o.d., Dow Corning, Midland, Michigan). The tail of the mediastinal lymph node was ligated at the free margin of the inferior pulmonary ligament to decrease the contribution of nonpulmonary lymph. The catheter was exteriorized, and the chest wall was closed.

To resolve pulmonary atelectasis, which often developed during surgery and study, the lungs were briefly inflated every 30–60 minutes with peak airway pressures of 50 cm water. An external heater and drapes maintained the pulmonary artery temperature at 38.5–40.0°C.

Hemodynamic and Airway Measurements

SAP, central venous pressure (CVP), PAP, PAOP, and HR were monitored continuously using calibrated pressure transducers (model 1280C, Hewlett-Packard, Palo Alto, California) zeroed at left atrial level and an eight-channel recorder (model 7758B, Hewlett-Packard). Mean cardiac output was determined by thermodilution as the mean of three measurements using 5-ml injections of 0°C LR solution and a cardiac output computer (model COC 4000, Electra-catheter). Cardiac index was derived by dividing cardiac output (milliliters per minute) by the animal's body weight (kilograms). Systemic and pulmonary vascular resistance indexes (SVRI and PVRI, respectively) were calculated and...
Table 1. Treatment Groups in This Study

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment and dose</th>
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<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>rhTNFa (25 µg/kg, three sheep; 75 µg/kg [1], 100 µg/kg [1], 150 µg/kg [1])</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Meclofenamate (10 mg/kg) + rhTNFa (25 µg/kg)</td>
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<tr>
<td>3</td>
<td>4</td>
<td>rhLT (25 µg/kg)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Meclofenamate alone (10 mg/kg)</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>None (control)</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Escherichia coli LPS (1 µg/kg)</td>
</tr>
</tbody>
</table>

rhTNFa, recombinant human tumor necrosis factor alpha; rhLT, recombinant human lymphotixin; LPS, lipopolysaccharide.

t=0 minutes) to establish stable physiological baseline measurements. During this period, LR was infused at 15 ml/kg/hr to replace fluid and electrolyte losses due to orogastric suction and extravascular fluid shifts due to surgery and anesthesia.

Six groups of sheep were studied (Table 1). Group 1 sheep (n=6) were infused with increasing doses of rhTNFa (Genentech, South San Francisco, California). rhTNFa was supplied in aqueous solution at a concentration of 0.5 mg/ml with specific activity on murine fibroblasts of 5.2×10⁵ IU/mg. The endotoxin content was measured at less than 0.018 ng/mg rhTNFa by limulus amoebocyte lysate assay. rhTNFa was stored at −20°C until 30 minutes before infusion. From t=0 to t=5 minutes, we infused one of the following doses of rhTNFa: 25, 75, 100, or 150 µg/kg. With the supportive therapy described below, no significant differences of systemic and pulmonary hemodynamics were measured during the first 4 hours after challenge in sheep given either 25 µg/kg or those given 75–150 µg/kg; thus, these results were pooled and are presented as rhTNFa 25–150 µg/kg rhTNFa (group 1). A dose of 10 µg/kg rhTNFa was given to two additional sheep not included in group 1; results from these sheep are discussed separately. This range of doses (10–150 µg/kg) was chosen because the estimated peak rhTNFa concentrations in sheep blood (8–120 nM/l) overlapped the levels (1–50 nM/l) measured in rabbits given LPS and dogs given rhTNFa. In three sheep given 75, 25, and 10 µg/kg rhTNFa, respectively, the rhTNFa concentrations in selected lymph and arterial plasma samples were assayed by ELISA (enzyme-linked immunosorbent assay) (courtesy of Dr. Anthony Cerami, Laboratory of Medical Biochemistry, Rockefeller University, New York, New York and Dr. Stephen F. Lowry, Laboratory of Surgical Metabolism, Cornell University, Ithaca, New York). The lower limit of detection of this assay was 34 pg/ml (approximately 0.002 nM/l).

Because rhTNFa infusion caused prostacyclin release, group 2 sheep (n=3) were pretreated with a meclofenamate infusion (Warner Lambert, Ann Arbor, Michigan). 10 mg/kg dissolved in 3 ml ethanol and 150 ml LR solution, from t=−5 to t=0
minutes, and then given 25 μg/kg rhTNFα over the next 5 minutes. A dose of 25 μg/kg, rather than 10 μg/kg, rhTNFα was infused because it was the lowest dose that caused a 25% or greater decrease of SAP during the first 3 hours of study. The endotoxin content of meclofenamate was measured by limulus amebocyte lysate assay to be approximately 0.2 ng/mg meclofenamate (limulus amebocyte lysate assay performed by Associates of Cape Cod, West Falmouth, Massachusetts).

To contrast the effects of equal weights of rhTNFα with rhLT, group 3 sheep (n=4) received rhLT (specific activity of 1.2×10^8 U/mg, Genentech), 25 μg/kg dissolved in 10 ml LR solution and infused from t=0 to t=5 minutes. The endotoxin content of rhLT was 0.062 ng/mg rhLT. Group 4 sheep (n=2) received meclofenamate alone (10 mg/kg). Group 5 sheep (n=4) served as controls; they underwent all the surgical procedures but received no drug treatment. All infusions were given through the femoral vein catheter. The maximum amount of contaminant endotoxin injected with the drug preparations into each sheep was 0.003 ng/kg in group 1, 0.002 ng/kg in group 3, and 2 ng/kg in groups 2 and 4. Infusion of 2 ng/kg Serratia marcescens LPS does not cause detectable pulmonary vascular responses or leukopenia in sheep. To contrast the physiological effects of rhTNFα and rhLT with those of E. coli LPS, 11 sheep (group 6) were prepared in a similar fashion to the sheep given the cytokines. E. coli 0111B4:4 LPS (1 μg/kg, single lot, Westphal method, Difco, Detroit, Michigan) was then infused from t=0 to t=5 minutes. Seven sheep were studied before the TNF study was started; four sheep were studied during the TNF study to confirm the consistency of our technique and results. The measurements in these LPS groups were statistically similar; thus, the LPS data are grouped together.

After the onset of study, LR was initially infused at 4–5 ml/kg/hr. Fluid transfusion was subsequently increased, if necessary, to maintain mean SAP greater than 50 mm Hg and PAOP at baseline (t=0 minutes) levels ±2 mm Hg. The volume of infused fluid was recorded hourly. Vasopressor therapy was not given. All groups of sheep survived at least 4 hours, and the majority were studied for 6 hours after initiation of the study (t=0 to t=240–360 minutes). The studies using 150 and 75 μg/kg rhTNFα were terminated after 4 hours due to our inability to maintain SAP greater than 50 mm Hg despite rapid volume transfusion. A third study, infusion of 25 μg/kg rhTNFα, was terminated after 4 hours due to technical problems with monitoring.

Before the animals were killed, 4 ml blood was withdrawn into EDTA-containing tubes and frozen. Each animal was killed with intravenous phenobarbital and potassium chloride. The lungs were excised, passively drained of blood for several minutes, double wrapped in plastic bags, and stored at −20° C. Lung extravascular water content was determined after thawing the lungs. The right and left lungs were separated and dissected to remove bronchi greater than 5 mm diameter. The percentage of lung extravascular water content was determined using the method of Peterson et al.24

Statistical Analysis

All data are expressed as mean±SEM. Changes of each parameter over time within and between the study groups were evaluated with multivariate analysis of variance for repeated measures as implemented in the SAS statistical programs (version 5.16, SAS Institute, Cary, North Carolina). Planned comparisons between mean values at baseline (before drug treatment) and at subsequent times within each treatment group were performed using the F test and the two-tailed t test while contrasting the responses of the control group and the selected study group. Baseline values in the control and treatment groups were compared using a one-way analysis of variance and t tests. Planned comparisons were tested individually at a 0.05 significance level. Post hoc comparisons within a selected variable were tested at a simultaneous 0.05 level according to Bonferroni's inequality. The Pearson product-moment correlation coefficient was used to measure the association between changes of selected biochemical and hemodynamic variables.

Results

Systemic Hemodynamics and Fluid Transfusion Requirements

Within 15–30 minutes of rhTNFα infusion, both SAP (Figure 1, top) and SVRI decreased profoundly when compared with the other treatment groups. SVRI decreased from 805±95 to 534±63 mm Hg×min/1 by 30 minutes (p<0.01 differs from baseline) and remained near this level for the duration of study (594±138 mm Hg×min/l at 6 hours). Vigorous fluid transfusion, up to five times the average rate given to untreated control sheep, was required to maintain SAP above 50 mm Hg and PAOP at baseline values ±2 mm Hg (Table 2). Sheep given 10 μg/kg rhTNFα experienced less decrease of SAP and SVRI (data not shown, p<0.05) and did not require an increased rate of fluid infusion (Table 2).

Meclofenamate pretreatment completely prevented the decrease of SVRI caused by a subsequent infusion of 25 μg/kg of rhTNFα (SVRI was 823±35 mm Hg×min/l at 0 minutes and was never less than 759±68 mm Hg×min/l). Meclofenamate pretreatment also attenuated (p<0.05) the early (<2 hours) decrease of SAP caused by 25 μg/kg rhTNFα, but not the later systemic hypotension (SAP was 53±4 mm Hg at 6 hours, p=0.005 differs from baseline). Average fluid transfusion rates for meclofenamate+rhTNFα sheep were approximately 50% less than the average fluid transfusion rate required by sheep given 25 μg/kg rhTNFα without a cyclooxygenase inhibitor (Table 2, p<0.05). Infusion of meclofenamate alone did not change SAP, SVRI, or fluid transfusion rates.
In contrast to sheep given rhTNFα, sheep given 1 μg/kg E. coli LPS had a more gradual decrease of SAP and markedly reduced fluid transfusion requirements. Furthermore, SVRI did not decrease at any time (data not shown).

In sheep given 25 μg/kg rhLT, both SAP and SVRI decreased 3–4 hours after infusion. Whereas SAP recovered toward baseline level by 6 hours, the decrease of SVRI persisted (SVRI fell from a baseline value of 902±99 to 672±55 mm Hg×min/l at 4 hours and 489±58 mm Hg×min/l at 6 hours, p<0.01 differs from baseline). SVRI of sheep given rhLT was comparable to the concomitant SVRI at 6 hours in sheep given 25–150 μg/kg rhTNFα (594±138 mm Hg×min/l). The fluid transfusion rates in sheep given rhLT were less than half the rates required after 25 μg/kg rhTNFα (Table 2).

HR and CVP did not change significantly in any study group (data not shown). Fluid transfusion maintained a stable PAOP at baseline ±2 mm Hg in all treatment groups (data not shown).

Pulmonary Hemodynamics and Cardiac Index

LPS infusion caused a biphasic increase of PAP (Figure 1, middle), which nearly doubled to 30 mm Hg at 30 minutes, returned toward baseline value after 2 hours, then gradually increased again to approximately 26 mm Hg after 6 hours. Changes of PVRI paralleled the changes of PAP: PVRI quadrupled at 30 minutes (increasing from 64±7 to 253±52 mm Hg×min/l, p<0.001), rapidly decreased to near-baseline levels during the next 1–2 hours, then increased to approximately three times baseline value after 6 hours (p<0.001, value differs from baseline data not shown).

In contrast to LPS-treated sheep, rhTNFα infusion caused only a minor increase of PAP during the first hour (Figure 1, middle, p<0.05 increase of PAP in rhTNFα-treated sheep differs from concomitant LPS-induced increase of PAP). PAP in rhTNFα sheep remained elevated at 6 hours. We also measured a gradual increase of PVRI which became statistically significant only after 6 hours (increasing from 86±13 to 239±44 mm Hg×min/l, p<0.001).
Cardiac index decreased concomitantly with the significant increase of PVRI, but not of PAP, which decrease in either control or rhLT-treated sheep. Decrease of SAP in sheep given either LPS or 4-5 hours after the decrease of SAP, whereas mate+rhTNF α-treated sheep decreased by 40-50% the remainder of the study. Notably, the decrease of SAP in sheep given rhTNF α alone. PAP increased in sheep given rhTNF α but were comparable to the values measured rhTNF α-induced increase of PAP (Figure 2); whereas in sheep given rhTNF α but were comparable to the values measured after LPS infusion (p<0.05). In contrast to the LPS-treated sheep, the L/P ratio in the rhTNF α sheep slowly increased by 16% after 3-4 hours (p<0.05), then returned to baseline value during the following 2 hours (data not shown). Notably, infusion of 10 μg/kg rhTNF α produced a marked increase of the L/P ratio (from 0.70±0.01 to t=0 minutes to a peak value of 0.88±0.01, p<0.05). This dose of rhTNF α increased Ql in both sheep (from 2.5±0.6 to a peak flow of 5.6±1.3 ml/15 min).

Meclofenamate pretreatment attenuated (p<0.05) the small increases of Ql and Clp during the first 4 hours after rhTNF α infusion but did not modify either the subsequent increases of Ql and Clp (meclofenamate+rhTNF α data not shown) or the increase of the L/P ratio.

rhLT infusion caused a 15% increase of the L/P ratio after 3 hours, which, in contrast to the L/P ratio in rhTNF α-treated sheep, remained elevated at 6 hours. Ql and Clp after rhLT increased later than in sheep given rhTNF α but were comparable to the values in rhTNF α-treated sheep at 6 hours.

Biochemical Studies  
6-k-PGF α measurements. Concomitant with the decreases of SAP and SVRI, both lung lymph and arterial plasma levels of 6-keto-PGF α increased markedly within 15-30 minutes after rhTNF α infusion and remained elevated throughout the study with lymph levels (Figure 3) consistently two to five times higher than the simultaneous plasma levels (plasma data not shown). Infusion of 150 μg/kg rhTNF α into one sheep caused peak lymph and arterial plasma 6-k-PGF α levels of nearly 30 ng/ml and 6 ng/ml, respectively. Infusion of 10 μg/kg rhTNF α (n=2 sheep) produced smaller peak 6-k-PGF α levels (from 0.53±0.04 to 1.71±0.23 ng/ml in lymph and from 0.25±0.03 to 0.58±0.07 ng/ml in

Table 2. Average Rate of Lactated Ringer’s Solution Infusion After t =0 Minutes to Maintain Mean Systemic Arterial Pressure Above 50 mm Hg and Pulmonary Artery Balloon Occlusion Pressure at Baseline Value±2 mm Hg

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ml/kg/hr</th>
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<tbody>
<tr>
<td>rhTNF α (25–150 μg/kg)</td>
<td>(n=6) 32.2±8.7*</td>
</tr>
<tr>
<td>rhTNF α (25 μg/kg)</td>
<td>(n=3) 25.9±2.4*</td>
</tr>
<tr>
<td>rhTNF α (10 μg/kg)</td>
<td>(n=2) 7.1±3.2</td>
</tr>
<tr>
<td>Meclofenamate+rhTNF α (25 μg/kg)</td>
<td>(n=3) 12.8±0.9*†</td>
</tr>
<tr>
<td>rhLT (25 μg/kg)</td>
<td>(n=4) 10.8±0.7*†</td>
</tr>
<tr>
<td>Escherichia coli LPS (1 μg/kg)</td>
<td>(n=4) 15.5±2.6*†</td>
</tr>
<tr>
<td>Meclofenamate alone (10 mg/kg)</td>
<td>(n=2) 4.0±0.3</td>
</tr>
<tr>
<td>Control</td>
<td>(n=4) 6.5±2.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. n=number of sheep in each group. rhTNF α recombinant human tumor necrosis factor alpha; rhLT, recombinant human lymphotoxin.

*p<0.05 significant difference compared with control group.

†p<0.05 significant difference compared with value in sheep treated with greater than 10 μg/kg rhTNF α and which was comparable to the LPS-induced PVRI at 6 hours.

Meclofenamate pretreatment augmented both PVRI (p<0.05) and PAP (p<0.05) at 60 minutes to values greater than the concomitant PVRI and PAP in sheep given rhTNF α alone. Meclofenamate+rhTNF α treatment increased PVRI from 66±13 to 160±29 mm Hg/min/l after 1 hour (p<0.01 differs from baseline), whereas PVRI did not change at 60 minutes in sheep given rhTNF α alone. PAP increased in meclofenamate+rhTNF α sheep from 15±0 to 24±1 mm Hg at 60 minutes (p<0.001 differs from baseline). In contrast, PAP increased minimally in sheep given rhTNF α alone (Figure 1, middle). However, after 6 hours, PAP and PVRI in meclofenamate+rhTNF α sheep were comparable to values measured in sheep given rhTNF α alone.

rhLT infusion induced a minor increase of PAP only during the first hour which was comparable to the rhTNF α-induced increase of PAP (Figure 2); however, PVRI did not change at any time.

Cardiac index (Figure 1, bottom) in LPS-treated sheep decreased briefly by approximately 30% during the acute LPS-induced pulmonary vasoconstriction (at 30–45 minutes), recovered after 1 hour, then decreased more gradually over 4–6 hours. Cardiac index decreased gradually in rhTNF α-treated sheep to approximately 50–60% of baseline value at 6 hours. Cardiac index in meclofenamate+rhTNF α-treated sheep decreased by 40–50% (p<0.05) at 2 hours and remained at this level for the remainder of the study. Notably, the decrease of cardiac index in rhTNF α-treated sheep occurred 4–5 hours after the decrease of SAP, whereas cardiac index decreased concomitantly with the decrease of SAP in sheep given either LPS or meclofenamate+rhTNF α. Cardiac index did not decrease in either control or rhLT-treated sheep.

Infusing meclofenamate alone caused a small but significant increase of PVRI, but not of PAP, which was accompanied by a slow decrease of cardiac index (from 134±8 at 0 minutes to 92±11 ml×kg/min at 6 hours, p<0.05).

Airway Pressure

Peak airway pressure increased 30 minutes after LPS infusion and remained elevated for most of the study (Table 3), whereas it increased only after 3 hours in sheep given rhTNF α. In addition, several of the LPS-treated sheep, but none of the rhTNF α-treated sheep, developed wheezing by the end of study. Airway pressure did not change in any of the other study groups.

Lung Lymph Dynamics (Figure 2)

Ql and Clp rates increased within 30 minutes after infusion of LPS and continued to rise to approximately three to four times baseline flow rates after 6 hours. The L/P ratio after LPS decreased by 13% after 2 hours (p<0.05, data not shown), then returned to baseline values during the next hour.

In sheep given rhTNF α Ql and Clp increased more slowly and to lower peak flow rates than those measured after LPS infusion (p<0.05). In contrast to the LPS-treated sheep, the L/P ratio in the rhTNF α sheep slowly increased by 16% after 3-4 hours (p<0.05), then returned to baseline value during the following 2 hours (data not shown). Notably, infusion of 10 μg/kg rhTNF α produced a marked increase of the L/P ratio (from 0.70±0.01 at t=0 minutes to a peak value of 0.88±0.01, p<0.05). This dose of rhTNF α increased Ql in both sheep (from 2.5±0.6 to a peak flow of 5.6±1.3 ml/15 min).

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rhLT infusion caused a 15% increase of the L/P ratio after 3 hours, which, in contrast to the L/P ratio in rhTNF α-treated sheep, remained elevated at 6 hours. Ql and Clp after rhLT increased later than in sheep given rhTNF α but were comparable to the values in rhTNF α-treated sheep at 6 hours.

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6-k-PGF α measurements. Concomitant with the decreases of SAP and SVRI, both lung lymph and arterial plasma levels of 6-keto-PGF α increased markedly within 15–30 minutes after rhTNF α infusion and remained elevated throughout the study with lymph levels (Figure 3) consistently two to five times higher than the simultaneous plasma levels (plasma data not shown). Infusion of 150 μg/kg rhTNF α into one sheep caused peak lymph and arterial plasma 6-k-PGF α levels of nearly 30 ng/ml and 6 ng/ml, respectively. Infusion of 10 μg/kg rhTNF α (n=2 sheep) produced smaller peak 6-k-PGF α levels (from 0.53±0.04 to 1.71±0.23 ng/ml in lymph and from 0.25±0.03 to 0.58±0.07 ng/ml in
Compared with sheep given rhTNFα, lung lymph 6-k-PGF₁α levels in LPS-treated sheep increased more slowly and returned more quickly to baseline values (Figure 3); arterial plasma 6-k-PGF₁α levels did not increase after LPS infusion. The peak lymph levels of 6-k-PGF₁α (1–6.2 ng/ml) after LPS infusion overlapped the peak lymph 6-k-PGF₁α levels (1.5–5.9 ng/ml) after infusion of 10–100 μg/kg rhTNFα (lymph 6-k-PGF₁α levels after 150 μg/kg rhTNFα are noted above). The decrease of SAP in LPS-treated sheep did not correlate with either lymph or arterial 6-k-PGF₁α levels.

Lymph and plasma levels of 6-k-PGF₁α did not change in the other study groups.

**Thromboxane B₂ measurements.** LPS infusion caused a marked elevation of TXB₂ levels in both lung lymph (Figure 4) and arterial plasma (data not shown), which peaked at 1 hour, concomitant with the acute increase of PAP (Figure 1, middle) and PVRI. Lymph TXB₂ levels increased from 1.5 to 9 ng/ml during the first hour, decreased to baseline values during the next several hours, and were consistently 1.5–3.5 times the simultaneous plasma TXB₂ levels.

In contrast to LPS-treated sheep, rhTNFα infusion caused a small increase of lymph TXB₂ levels at 15 and 30 minutes (Figure 4) whereas we measured a minor increase of plasma TXB₂ levels (<1 ng/ml) in rhTNFα-treated sheep at 3–4 hours. The peak lymph and plasma levels of TXB₂ did not correlate with the dose of rhTNFα infused. Plasma and lymph TXB₂ levels did not increase in the other study groups.

**rhTNFα measurements.** Assay of arterial plasma and lung lymph rhTNFα concentrations in three sheep given 10, 25, and 75 μg/kg, respectively, demonstrated that (Table 4) 1) lymph concentrations of approximately 1.9–4.9 nM/1 appeared within 15 minutes after rhTNFα infusion and peaked after 30–60 minutes at approximately 4.2–14.2 nM/1, 2) peak lung lymph concentrations in each of these sheep were less than one half the peak plasma concentrations, 3) rhTNFα levels remained measurable in both lymph and plasma after 4 hours of study.

**Additional Hematologic Studies**

In all sheep, infusion of LPS and all doses of the cytokines caused severe leukopenia within 30 minutes, which persisted throughout the study (Figure 5). The leukopenia developed more quickly after infusion of the cytokines than after LPS. Meclofenamate pretreatment did not modify the leukopenia. No transpulmonary arteriovenous leukocyte gradients were measured in any group. Hematocrit remained stable in all groups despite blood sampling and differences of fluid infusion rates. Plasma protein concentration gradually decreased 35–40% by the end of study in sheep given either rhTNFα or rhLT, by approximately 25% after meclofenamate + rhTNFα infusion, and by 20% after LPS infusion.
TABLE 3. Peak Airway Pressure at 0, 30, 60, 120, 240, and 360 Minutes After Treatment

<table>
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<th>Time (minutes)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
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<td>Airway pressure (cm H$_2$O)</td>
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<tr>
<td>rhTNF$_a$</td>
<td>16.3±1.2</td>
<td>17.0±1.3</td>
<td>17.5±1.5</td>
<td>18.0±2.3</td>
<td>26.3±4.0</td>
<td>24.3±0.3*</td>
</tr>
<tr>
<td>M+rhTNF$_a$</td>
<td>15.0±0.6</td>
<td>15.0±0.6</td>
<td>15.3±0.9</td>
<td>14.7±0.9</td>
<td>16.0±1.2</td>
<td>16.7±1.2</td>
</tr>
<tr>
<td>rhLT</td>
<td>16.5±1.0</td>
<td>16.8±0.9</td>
<td>17.3±1.1</td>
<td>16.5±0.9</td>
<td>18.7±2.7</td>
<td>17.7±1.2</td>
</tr>
<tr>
<td>LPS</td>
<td>19.6±2.0</td>
<td>23.6±3.0*</td>
<td>21.7±2.4*</td>
<td>22.3±2.8</td>
<td>23.2±2.7*</td>
<td>29.3±1.8*</td>
</tr>
<tr>
<td>Control</td>
<td>15.5±1.6</td>
<td>14.5±1.6</td>
<td>14.5±0.6</td>
<td>15.5±1.0</td>
<td>14.5±0.5</td>
<td>15.5±0.5</td>
</tr>
</tbody>
</table>

Treatments: recombinant human tumor necrosis factor alpha (rhTNF$_a$) 25–150 μg/kg; M+rhTNF$_a$, meclofenamate (10 mg/kg)+rhTNF$_a$, 25 μg/kg; recombinant human lymphotxin (rhLT) 25 μg/kg; LPS, Escherichia coli lipopolysaccharide 1 μg/kg; Peak airway pressure did not change in sheep given meclofenamate alone (10 mg/kg).

*p<0.05 significant change of variable compared with time-matched change in control group.

#Blood Gas Studies#
Arterial Po$_2$ decreased profoundly and Qs/Qt increased markedly by the end of study in four of the six sheep given rhTNF$_a$ (Table 6). Arterial pH decreased by the end of study in all rhTNF$_a$-treated sheep and less in sheep given meclofenamate (p<0.05, rhTNF$_a$ vs. meclofenamate+rhTNF$_a$). Changes of Qs/Qt, arterial Po$_2$ and pH were not measured in the other study groups. Arterial Pco$_2$ was stable in all except two sheep given rhTNF$_a$ (data not shown).

#Pathology Studies#
Gross examination of the lungs at the end of study revealed that, despite frequent lung inflations to minimize atelectasis, sheep treated with 75–150 μg/kg rhTNF$_a$ had widespread atelectasis of dependent lung regions. In contrast, one of the three sheep given 25 μg/kg rhTNF$_a$ had moderate atelectasis of the dependent lung, while the other two sheep had minor atelectasis. None of the other lungs demonstrated visible atelectasis.

Calculations of lung extravascular water content did not differ between the control group and any other study group (all ranged from 77.1% to 81.1% water by weight). These results are similar to the lung extravascular water content values measured previously in our laboratory in control sheep (78.1±0.6%) and in awake sheep 2 hours after infusion of E. coli 0111:B4 LPS, 1 μg/kg.

#Discussion#
Our study compared the effects of intravenous infusions of rhTNF$_a$, rhLT, and E. coli LPS into sheep to measure qualitative differences of physio-
TABLE 4. Selected Lymph and Arterial Plasma Concentrations of rhTNFα

| Dose rhTNFα infused | Concentration (nM/l) | Time | | |
|---------------------|----------------------|------|------|------|------|------|
|                     | L                    | A    | L    | A    | L    | A    |
| 75 µg/kg            | 4.9                  | >25  | 14.2 (60') | >25  | 4.7  | >25  |
| 25 µg/kg            | 1.7                  | 8.6  | 3.5 (60-120') | >25  | 2.2  | 4.8  |
| 10 µg/kg            | 1.9                  | 14.6 | 4.1 (30') | 14.6 | 0.4  | 0.7  |

rhTNFα recombinant human tumor necrosis factor alpha; L, lung lymph; A, arterial plasma. Times inside parentheses are the times the samples were obtained. Levels were measured from one sheep at each dose. The lower limit of detection by enzyme-linked immunosorbent assay was 34 pg/ml (approximately 0.002 nM/l).

logical and eicosanoid responses to these agents. Our study provides evidence that eicosanoid mediators contribute importantly to physiological responses caused by infusion of rhTNFα, but not after the same dose of rhLT. We also learned that the pulmonary and systemic hemodynamic and eicosanoid responses to LPS infusion differ from those produced by rhTNFα.

Infusion of 25-150 µg/kg rhTNFα caused rapid and marked increases of both lung lymph and plasma levels of the stable and inactive prostacyclin metabolite, 6-k-PGFia. These increases were associated with rapid decreases of SAP and SVRI. Cyclooxygenase blockade with meclofenamate prevented the increases of 6-k-PGFia levels, the decrease of SVRI, and attenuated the early (<2 hours), but not the late (4-6 hours), decrease of SAP caused by infusing 25 µg/kg rhTNFα. Meclofenamate reduced the fluid transfusion rate required to maintain SAP above 50 mm Hg and PAOP near prestudy levels. These results suggest that the early decrease of SAP after rhTNFα infusion is due primarily to increased concentrations of eicosanoid vasodilators whereas the late decrease of SAP may partly be due to increased systemic vascular permeability and loss of intravascular fluid and solutes.

In contrast to these results, lymph and plasma 6-k-PGFia levels in sheep given 1 µg/kg of E. coli LPS increased slightly later than in the rhTNFα sheep and returned more quickly to baseline values. SAP also decreased later in sheep treated with LPS than in sheep given rhTNFα. Whereas the initial decrease of SAP correlated with the increase of lymph 6-k-PGFia levels in rhTNFα-treated sheep, these variables did not correlate in sheep given LPS. Notably, SVRI did not decrease after LPS infusion; SVRI did not decrease in other studies of awake sheep infused with 1 µg/kg of E. coli LPS. Thus, LPS and rhTNFα cause differing patterns of prostacyclin release, systemic vasodilation, and systemic hypotension.

The dose of LPS that we examined caused briefer prostacyclin release and less systemic vasodilation than did 25-150 µg/kg rhTNFα, yet, this dose of LPS caused greater release of thromboxane into lung lymph and plasma, pulmonary vasoconstriction, and greater pulmonary artery hypertension during the first hour of study. Acute pulmonary hypertension in sheep has been reported after infusing a wide range of doses (0.07-1.33 µg/kg) of E. coli 0127:B8 LPS; the LPS-induced pulmonary hypertension can be blocked by pretreatment with cyclooxygenase inhibitors. Although meclofenamate pretreatment blocked prostacyclin release and reduced fluid transfusion requirements, it did not modify the increase of the L/P ratio, lung lymph flow, or protein clearance 4-6 hours after rhTNFα infusion. The increase of lung lymph flow in sheep given 25-150 µg/kg rhTNFα may be partly due to major fluid transfusion, which was associated with a decreased plasma protein concentration. However, fluid transfusion was not the sole determinant of the increased lymph flow: the two sheep given 10 µg/kg rhTNFα did not require significant fluid transfusion (Table 2) and had stable plasma protein concentrations, but both of these sheep demonstrated an increased L/P ratio and lung lymph flow. Thus, rhTNFα may have caused a minor increase of pulmonary microvascular permeability.

The effects of LPS and rhTNFα on lung lymph measurements contrast in several ways. First, LPS caused greater early increases of Ql, Clp, and PAP than did rhTNFα. Cyclooxygenase blockade of sheep given LPS attenuates the early, acute pulmonary hypertension and the early, but not late, increases
Table 5. Total Plasma Protein Concentration

<table>
<thead>
<tr>
<th>Plasma Protein (gm/dl)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>End (240–360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNFα (25-150 µg/kg)</td>
<td>5.4±0.2</td>
<td>4.9±0.2*</td>
<td>4.6±0.2*</td>
<td>4.5±0.2</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>M+rhTNFα (25 µg/kg)</td>
<td>4.8±0.2</td>
<td>4.4±0.2</td>
<td>4.3±0.3</td>
<td>4.2±0.3</td>
<td>3.6±0.1*</td>
</tr>
<tr>
<td>rhLT (25 µg/kg)</td>
<td>5.0±0.1</td>
<td>4.6±0.2</td>
<td>4.5±0.2</td>
<td>4.5±0.2</td>
<td>3.1±0.2*</td>
</tr>
<tr>
<td>LPS (1 µg/kg)</td>
<td>4.6±0.4</td>
<td>4.4±0.4</td>
<td>4.3±0.3</td>
<td>4.0±0.3</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>Control</td>
<td>5.1±0.3</td>
<td>4.9±0.4</td>
<td>4.9±0.4</td>
<td>4.5±0.7</td>
<td>4.8±0.3</td>
</tr>
</tbody>
</table>

rhTNFα, recombinant human tumor necrosis factor alpha; M+rhTNFα, meclofenamate (10 mg/kg)+rhTNFα; rhLT, recombinant human lymphotoxin; LPS, Escherichia coli lipopolysaccharide. Meclofenamate alone (10 mg/kg) did not change the plasma protein concentration.

Table 6. Selected Arterial Blood Gas and Pulmonary Shunt Data

<table>
<thead>
<tr>
<th>Arterial PO2 (mm Hg)</th>
<th>Time (minutes)</th>
<th>End (240–360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNFα (75-150 µg/kg)</td>
<td>(n=3)</td>
<td>473±18</td>
</tr>
<tr>
<td>rhTNFα (25 µg/kg)</td>
<td>(n=3)</td>
<td>510±37</td>
</tr>
<tr>
<td>M+rhTNFα (25 µg/kg)</td>
<td>(n=3)</td>
<td>507±12</td>
</tr>
<tr>
<td>rhLT (25 µg/kg)</td>
<td>(n=3)</td>
<td>461±44</td>
</tr>
<tr>
<td>LPS (1 µg/kg)</td>
<td>(n=5)</td>
<td>527±58</td>
</tr>
<tr>
<td>Meclofenamate alone (10 mg/kg)</td>
<td>(n=2)</td>
<td>397±68</td>
</tr>
<tr>
<td>Control</td>
<td>(n=4)</td>
<td>461±41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arterial pH</th>
<th>Time (minutes)</th>
<th>End (240–360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNFα (75-150 µg/kg)</td>
<td>7.45±0.06</td>
<td>7.23±0.04*†</td>
</tr>
<tr>
<td>rhTNFα (25 µg/kg)</td>
<td>7.39±0.0</td>
<td>7.20±0.05*†</td>
</tr>
<tr>
<td>M+rhTNFα</td>
<td>7.48±0.03</td>
<td>7.37±0.03</td>
</tr>
<tr>
<td>rhLT</td>
<td>7.45±0.03</td>
<td>7.42±0.05</td>
</tr>
<tr>
<td>LPS</td>
<td>7.50±0.02</td>
<td>7.46±0.02</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>7.35±0.03†</td>
<td>7.44±0.01*</td>
</tr>
<tr>
<td>Control</td>
<td>7.45±0.02</td>
<td>7.43±0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Qs/Qr (percent cardiac output)</th>
<th>Time (minutes)</th>
<th>End (240–360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNFα (75-150 µg/kg)</td>
<td>14±1</td>
<td>40±5*†</td>
</tr>
<tr>
<td>rhTNFα (25 µg/kg)</td>
<td>15±3</td>
<td>28±17</td>
</tr>
<tr>
<td>M+rhTNFα</td>
<td>15±3</td>
<td>8±2</td>
</tr>
<tr>
<td>rhLT</td>
<td>18±2</td>
<td>19±1*†</td>
</tr>
<tr>
<td>LPS</td>
<td>16±2</td>
<td>15±5</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>19±4</td>
<td>12±1*</td>
</tr>
<tr>
<td>Control</td>
<td>17±4</td>
<td>11±2</td>
</tr>
</tbody>
</table>

rhTNFα, recombinant human tumor necrosis factor alpha; M+rhTNFα, meclofenamate (10 mg/kg)+rhTNFα; rhLT, recombinant human lymphotoxin; LPS, Escherichia coli lipopolysaccharide; Qs/Qr, lung shunt fraction as percent of right cardiac output. Results from sheep treated with rhTNFα are divided into two groups (75–150 µg/kg). All other data are expressed as mean±SEM. None of these variables changed in sheep treated with 10 µg/kg rhTNFα.

*p<0.05 significant change of variable compared with change in control group.

| p<0.05 significant difference compared with time-matched value in control group.
LPS infusion, perhaps in conjunction with other cytokines.

Infusion of 25–150 μg/kg rhTNFα caused less thromboxane release and acute pulmonary vasoconstriction, a smaller increase of pulmonary artery pressure, and more gradual increase of airway pressure than did LPS challenge. However, sheep given 75–150 μg/kg rhTNFα had marked atelectasis, severe right-to-left lung shunt, and marked hypoxemia at 6 hours, although these changes did not develop consistently in sheep given lower doses of rhTNFα. Atelectasis, increased shunt, and hypoxemia did not occur in LPS-treated sheep, which contrasts with the LPS-induced hypoxemia in other sheep studies.17 Calculation of extravascular lung water content in our study demonstrates that, at the doses we studied, neither LPS nor rhTNFα caused major pulmonary edema. Notably, meclofenamate pretreatment attenuated both the fluid transfusion requirements and the increase of airway pressure in sheep given 25 μg/kg rhTNFα. Because sheep treated with 25 μg/kg rhTNFα did not consistently develop an increased shunt fraction or hypoxemia, we could not assess the effect of meclofenamate on these variables. Our results suggest that cyclooxygenase, and perhaps lipoxygenase products, as well as major fluid transfusion may contribute to airway closure and atelectasis, increased shunt fraction, hypoxemia, and increased airway pressure caused by infusion of rhTNFα. We do not know why meclofenamate acutely augmented the rhTNFα-induced increase of PAP and PVRI. In contrast, cyclooxygenase inhibitors attenuate both the acute LPS-induced decrease of dynamic airway compliance and the early increase of pulmonary artery pressure in sheep.17

Infusion of rhTNFα but not LPS, caused metabolic acidemia. Meclofenamate treatment attenuated this acidemia, which appears similar to indomethacin blocking rhTNFα-induced acidemia in rats.9 The precise cause of the acidemia is unknown. The ability of meclofenamate to ameliorate the acute rhTNFα-induced systemic hypotension and reduce fluid transfusion requirements in sheep suggests that eicosanoids contribute to systemic hypoperfusion and acidemia. Studies in which cyclooxygenase inhibitors were administered to animals prior to infusion of either rhTNFα or LPS17,21,25,30,34 support the hypothesis that eicosanoids may increase morbidity and mortality. Our results demonstrate that in addition to blocking release of thromboxane and prostacyclin, meclofenamate primarily attenuates the systemic vasodilation, initial systemic hypotension, and metabolic acidemia caused by rhTNFα infusion; cyclooxygenase blockade did not completely prevent the increased fluid transfusion requirement and the late systemic hypotension.

Infusion of 25 μg/kg rhLT did not cause increased plasma or lymph TXB2 or 6-k-PGF1α, levels, acute pulmonary hypertension, or pulmonary atelectasis. Infusing rhLT caused more gradual systemic vasodilation and hypotension than did 25 μg/kg rhTNFα, required markedly lower fluid transfusion rates, and did not cause acidemia. However, rhLT infusion increased the L/P ratio and caused small increases of QI and Clp after 6 hours which were statistically comparable to those induced by rhTNFα, (Figure 2). These results suggest that 6 hours after infusion, both rhLT and rhTNFα may cause a minor increase of lung microvascular permeability. The change is small and occurred concomitantly with a progressive reduction of plasma protein levels, making precise interpretation difficult. Because we measured plasma levels of rhTNFα but not rhLT, we cannot state with certainty that at an equivalent concentration, rhLT is a weaker stimulus for many of these changes, including prostacyclin release into plasma and lymph. However, sufficient quantities of rhLT were administered to cause marked leukopenia and systemic vasodilation at 6 hours.

The doses of rhTNFα infused into our sheep were weight-adjusted and derived from comparison with the peak serum TNFα, levels measured following infusion of 6–10 μg/kg E. coli 0127:B8 LPS into rabbits.13 In rabbits, detectable serum TNFα levels appear within 15 minutes after LPS infusion, peak after approximately 2 hours at 1–50 nM/l, and return to baseline levels after 5 hours. Thus, the rhTNFα levels in our study (Table 4) overlap those reported in rabbits after LPS infusion. Because the species-specific differences of TNFα, LT, and their receptors are incompletely defined, ideal comparisons of the effects of LPS with these cytokines in sheep would include studies that use sheep TNFα and LT, which are presently unavailable, rather than human recombinant TNFα and LT. In addition, measurements of plasma levels of cytokines produced by sheep, rather than rabbits, after LPS challenge should guide dose determinations of future ovine studies with TNFα and LT. Physiological responses caused by synergy between the recombinant cytokines and contaminating LPS in the cytokine preparations are likely to be minor, because LAL assay measured very low levels of LPS.

Comparison of simultaneous lung lymph and arterial plasma levels of 6-k-PGF1α after infusion of either LPS or rhTNFα reveals that lymph levels consistently exceeded plasma levels. These results suggest that the high lymph levels of prostacyclin do not increase solely by diffusion of plasma prostacyclin derived from extrapulmonary sources into lung interstitium; the lung produces large quantities of prostacyclin. A similar comparison of TXB2 levels suggests that LPS induces pulmonary production of thromboxane. Both pulmonary intravascular macrophages31 and pulmonary vascular endothelium may contribute importantly to the release of thromboxane32 and prostacyclin, respectively. Other investigators have also suggested that LPS induces pulmonary release of thromboxane and prostacyclin in sheep.27,30
Differences between lymph and plasma 6-k-PGF\(_{1\alpha}\) levels in sheep given either LPS or rhTNF\(_{a}\) may be due to multiple factors. The delay between LPS stimulation of macrophages and subsequent TNF\(_{a}\) release probably contributes to the one hour delay before lymph 6-k-PGF\(_{1\alpha}\) levels increase significantly; however, this does not explain the decline of 6-k-PGF\(_{1\alpha}\) levels in LPS-treated sheep to baseline values after 4 hours. Lung lymph 6-k-PGF\(_{1\alpha}\) levels were elevated throughout the study after rhTNF\(_{a}\) infusion. The doses of rhTNF\(_{a}\) we infused may produce higher plasma levels of TNF\(_{a}\) than would be produced by infusion of 1 \(\mu g/kg\) LPS. In addition, the functional half-lives of rhTNF\(_{a}\) and LPS may differ. It is also possible that LPS injures endothelial cells, an important source of prostacyclin, to such an extent that prostacyclin synthesis decreases.

In summary, we discovered major differences between the ovine responses to infusion of 1 \(\mu g/kg\) E. coli LPS, 25–150 \(\mu g/kg\) rhTNF\(_{a}\), and 25 \(\mu g/kg\) rhLT. Whereas both LPS and 25–150 \(\mu g/kg\) rhTNF\(_{a}\) acutely caused severe leukopenia and, after 6 hours, comparably increased PAP and PVR\(_{1}\), rhTNF\(_{a}\) caused more rapid and prolonged prostacyclin release into lung lymph and plasma, profound systemic vasodilation, and more rapid systemic hypotension than did LPS. LPS acutely caused greater thromboxane release into lung lymph and plasma, pulmonary vasoconstriction, and greater acute pulmonary hypertension. In addition, LPS caused a greater increase of pulmonary microvascular permeability after 6 hours than did rhTNF\(_{a}\), which may have induced only a minor permeability increase.

rhLT infusion caused marked acute leukopenia and progressive systemic vasodilation but few of the other changes measured after challenge with either LPS or rhTNF\(_{a}\). By studying doses of rhTNF\(_{a}\), rhLT, and LPS that caused some comparable physiological responses in the sheep, we identified several important differences of response.

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References


**KEY WORDS** • prostacyclin • thromboxane • monokines • cytokines • endotoxin • acute lung injury
Effects of recombinant human tumor necrosis factor alpha, lymphotoxin, and Escherichia coli lipopolysaccharide on hemodynamics, lung microvascular permeability, and eicosanoid synthesis in anesthetized sheep.

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