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/monocytes and natural killer cells, after activation by gram-negative bacteria, viruses, parasites, and lipo-polysaccharide (LPS). TNFα can induce the release of other inflammatory mediators, including interleukin-6–9 and eicosanoids such as prostaglandin E2,5 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

<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>Clp</td>
<td>Lung lymph transvascular protein clearance</td>
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<td>HR</td>
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<td>L/P</td>
<td>Ratio of lung lymph protein/arterial plasma protein</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>LR</td>
<td>Lactated Ringer’s solution</td>
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<tr>
<td>M</td>
<td>Meclofenamate</td>
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<td>PAOP</td>
<td>Pulmonary artery balloon occlusion pressure</td>
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<td>PAP</td>
<td>Mean pulmonary artery pressure</td>
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<td>PVRI</td>
<td>Pulmonary vascular resistance index</td>
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<tr>
<td>QI</td>
<td>Lymph flow</td>
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<tr>
<td>Qs/Qr</td>
<td>Lungs shunt fraction, as percent of right cardiac output</td>
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<td>rhTNFα</td>
<td>Recombinant human tumor necrosis factor alpha</td>
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<tr>
<td>SAP</td>
<td>Mean systemic arterial pressure</td>
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<tr>
<td>6-k-PGF1α</td>
<td>6-Keto-prostaglandin F1α</td>
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<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
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<td>TXB2</td>
<td>Thromboxane B2</td>
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- 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 biosay 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 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).

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 with 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 arterial 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).

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 CO 4000, Electra-cather). 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
adjusted for body weight using standard formulae: 
SVRI = (SAP−CVP)/cardiac index and PVRI = 
(PAP−PAOP)/cardiac index. Cardiac index, SVRI, 
and PVRI were measured every 30 minutes through-
out the study, except during the initial 1-hour period 
(t=0–60 minutes) when they were measured every 
15 minutes.

Airway pressure was monitored continuously 
using a pressure transducer (model 267BC, Hewlett-
Packard) connected to a side port at the proximal 
end of the endotracheal tube. Airway pressure was 
zeroed to atmospheric pressure.

Hematologic Studies

At selected times, simultaneous arterial and mixed 
venous blood samples (4 ml each) were aspirated 
into plastic syringes and immediately injected into 
iced, glass tubes containing 50 µl of 15% EDTA 
(Terumo Medical, Elkton, Maryland) and 100 µg 
indomethacin. Following measurement of hematocrit 
(Adams Autocrit Centrifuge, Clay Adams, Par-
sippany, New Jersey), arterial and venous leuko-
cyte counts (Coulter cell counter, model Z, Coulter 
Electronics, Hialeah, Florida), the samples were 
centrifuged at 2,000g for 10 minutes at 4°C. The 
plasma was aspirated, and the total protein concen-
tration was measured with a refractometer (model 
The samples were stored in polypropylene tubes at 
−70°C for later analysis of eicosanoid levels.

Arterial and mixed venous blood pH, PCO2, and 
PO2 were determined on 2 ml anerobically drawn, 
heparinized blood samples that were stored in ice 
until they were assayed (pH/Blood Gas Analyzer, 
model 170, Corning Medical, Medfield, Massachu-
setts). The right-to-left pulmonary shunt fraction 
(Qs/Qt) was calculated by a standard formula.19

Lymph Studies

Following the termination of surgery, lung lymph 
was collected every 15 minutes into iced, glass 
tubes containing EDTA (Terumo Medical) and 100 
µg indomethacin. Lung lymph flow (QL) was 
measured with a refractometer (model 10400A, American Optical, Buffalo, New York). The 
lymph samples were assayed by radioimmunoassay 
as described previously20,21 using antisera provided 
by Dr. L. Levine.22

Following termination of surgery, all animals 
were observed for a minimum of 2 hours (t=−120 to 
t=0 minutes) to establish stable physiological baseline 
measurements. During this period, LR was 
infused at 15 ml/kg/hr to replace fluid and electro-
lyte losses due to orogastric suction and extravas-
cular 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 5.2×1010 IU/mg. The endotoxin content was measured at less than 0.018 ng/mg rhTNFa by limulus amoebocyte lyase assay. rhTNFa was stored at −20°C until 3 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 sys-
temic and pulmonary hemodynamics were mea-
sured 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 
N/ml) measured in rabbits given LPS and 
dogs given rhTNFa.11 In three sheep given 75, 25, and 10 
µg/kg, the rhTNFa concentrations in selected lymph and arterial plasma samples 
were assayed by ELISA (enzyme-linked immunosorbent assay),14 (courtesy of Dr. Anthony 
Cerami, Laboratory of Medical Biochemistry, Rock-
efeller University, New York, New York and Dr. 
Stephen F. Lowry, Laboratory of Surgical Metab-
olism, 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 

| Table 1. Treatment Groups in This Study |
|---|---|---|
| Group | n | Treatment and dose |
| 1 | 6 | rhTNFa (25 µg/kg, three sheep; 75 µg/kg [1], 100 µg/kg [1], 150 µg/kg [1]) |
| 2 | 3 | Meclofenamate (10 mg/kg)+rhTNFa (25 µg/kg) |
| 3 | 4 | rhLT (25 µg/kg) |
| 4 | 2 | Meclofenamate alone (10 mg/kg) |
| 5 | 4 | None (control) |
| 6 | 11 | Escherichia coli LPS (1 µg/kg) |

rhTNFa, recombinant human tumor necrosis factor alpha; rhLT, recombinant human lymphotixin; LPS, lipopolysaccha-
ride.
minutes, and then given 25 μg/kg rhTNFa over the next 5 minutes. A dose of 25 μg/kg, rather than 10 μg/kg, rhTNFa 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 rhTNFa 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 rhTNFa 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 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 rhTNFa 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 rhTNFa, 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.25 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 rhTNFa 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/1 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 rhTNFa 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 rhTNFa (SVRI was 823±35 mm Hg×min/1 at 0 minutes and was never less than 759±68 mm Hg×min/1). Meclofenamate pretreatment also attenuated (p<0.05) the early (<2 hours) decrease of SAP caused by 25 μg/kg rhTNFa, 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+rhTNFa sheep were approximately 50% less than the average fluid transfusion rate required by sheep given 25 μg/kg rhTNFa, 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 rhTNF₀, 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 rhTNF₀ (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 rhTNF₀ (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, rhTNF₀ infusion caused only a minor increase of PAP during the first hour (Figure 1, middle, p<0.05 increase of PAP in rhTNF₀-treated sheep differs from concomitant LPS-induced increase of PAP). PAP in rhTNF₀ 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 of SAP in sheep given either LPS or the remainder of the study. Notably, the decrease Cardiac index decreased gradually in rhTNF α-treated sheep to approximately 50-60% of baseline treatment (at 30-45 minutes), recovered after 1 hour, then decreased more gradually over 4-6 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). 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 1α measurements. Concomitant with the decreases of SAP and SVRI, both lung lymph and arterial plasma levels of 6-keto-PGF 1α 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 1α 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 1α 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

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**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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<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</td>
<td>(n=3) 12.8±0.9*+</td>
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<tr>
<td>(25 µg/kg)</td>
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<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>
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</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 lyphotoxin.

*p<0.05 significant difference compared with control group.

+t=0 minutes to a peak value of 0.88±0.01, p<0.05).

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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).

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.
LUNG LYMPH FLOW

Figure 2. A: Lung lymph flow (Ql), expressed as milliliters per 15 minutes. B: Lung transvascular plasma protein clearance (Clp), expressed as milliliters per 15 minutes (calculated as Ql x ratio of [lung lymph protein]/[arterial plasma protein]. Meclofenamate treatment marginally attenuated (p<0.05) the small increase of Ql and Clp during the first four hours in sheep given 25 μg/kg rhTNFα but did not significantly modify the increase of these variables at 6 hours. For the sake of visual clarity, the data from sheep given meclofenamate + rhTNFα is not displayed in these figures. *p<0.05 significant change of variable in treatment group vs. time-matched change in control group. The apparent differences of Ql and Clp between these groups at t=0 minutes were not statistically significant. TNF-alpha, recombinant human tumor necrosis factor alpha; LPS, Escherichia coli lipopolysaccharide; LT, recombinant human lymphotoxin.

plasma, p<0.05 both values differ from baseline). The peak lymph levels of 6-k-PGF1α correlated with the infused dose of rhTNFα (r=0.92, p<0.001), whereas the increase of lymph 6-k-PGF1α levels, but not arterial 6-k-PGF1α levels, correlated with the reduction of SAP (r=-0.77, p<0.001) during the first hour after rhTNFα infusion (10–150 μg/kg).

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/l appeared within 15 minutes after rhTNFα infusion and peaked after 30–60 minutes at approximately 1.5–4 ng/ml. 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>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNF&lt;sub&gt;a&lt;/sub&gt;</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&lt;sub&gt;a&lt;/sub&gt;</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<sub>a</sub>) 25–150 μg/kg; M+rhTNF<sub>a</sub> meclofenamate (10 mg/kg)+rhTNF<sub>a</sub> 25 μg/kg; recombinant human lymphotoxin (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).

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

(Table 5). Plasma protein slowly decreased by 18% in sheep given 10 μg/kg rhTNF<sub>a</sub>.

Blood Gas Studies

Arterial Po<sub>2</sub> decreased profoundly and Qs/Qr increased markedly by the end of study in four of the six sheep given rhTNF<sub>a</sub> (Table 6). Arterial pH decreased by the end of study in all rhTNF<sub>a</sub>-treated sheep and less in sheep given meclofenamate (<i>p</i> < 0.05, rhTNF<sub>a</sub> vs. meclofenamate+rhTNF<sub>a</sub>). Changes of Qs/Qr, arterial Po<sub>2</sub> and pH were not measured in the other study groups. Arterial PCO<sub>2</sub> was stable in all except two sheep given rhTNF<sub>a</sub> (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<sub>a</sub> had widespread atelectasis of dependent lung regions. In contrast, one of the three sheep given 25 μg/kg rhTNF<sub>a</sub> 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%)<sup>26</sup> and in awake sheep 2 hours after infusion of E. coli 0111:B4 LPS, 1 μg/kg.<sup>27</sup>

Discussion

Our study compared the effects of intravenous infusions of rhTNF<sub>a</sub>, rhLT, and E. coli LPS into sheep to measure qualitative differences of physio-

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Lung lymph levels of 6-keto-prostaglandin F<sub>1α</sub> (6-keto-PGF<sub>1α</sub>). * <i>p</i> < 0.05 significant change of variable in treatment group vs. time-matched change in control group. Values at t=0 minutes do not differ between groups. TNF-alpha, recombinant human tumor necrosis factor alpha; MECLO, meclofenamate; LT, recombinant human lymphotoxin; LPS, Escherichia coli lipopolysaccharide.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Lung lymph thromboxane B<sub>2</sub> (TXB<sub>2</sub>) levels. * <i>p</i> < 0.05 significant change of variable in treatment group vs. time-matched change in control group. Values at t=0 minutes do not differ between groups. TNF-alpha, recombinant human tumor necrosis factor alpha; MECLO, meclofenamate; LT, recombinant human lymphotoxin; LPS, Escherichia coli lipopolysaccharide.
TABLE 4. Selected Lymph and Arterial Plasma Concentrations of rhTNF_

<table>
<thead>
<tr>
<th>Time</th>
<th>ARTERIAL LEUKOCYTE COUNTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (nM/l) in:</td>
</tr>
<tr>
<td></td>
<td>L  A  L  A  L  A</td>
</tr>
</tbody>
</table>
| Dose rhTNF_
infused |
| 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 |

rhTNF_
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 rhTNF_
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 rhTNF_
.

Infusion of 25–150 µg/kg rhTNF_
caused rapid and marked increases of both lung lymph and plasma levels of the stable and inactive prostacyclin metabolite, 6-k-PGF
. These increases were associated with rapid decreases of SAP and SVRI. Cyclooxygenase blockade with meclofenamate prevented the increases of 6-k-PGF
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 rhTNF_
. 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 rhTNF_
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-PGF
levels in sheep given 1 µg/kg of E. coli LPS increased slightly later than in the rhTNF_
sheep and returned more quickly to baseline values. SAP also decreased later in sheep treated with LPS than in sheep given rhTNF_
. Whereas the initial decrease of SAP correlated with the increase of lymph 6-k-PGF
levels in rhTNF_
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 rhTNF_
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 rhTNF_
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 rhTNF_
infusion. The increase of lung lymph flow in sheep given 25–150 µg/kg rhTNF_
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 rhTNF_
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, rhTNF_
may have caused a minor increase of pulmonary microvascular permeability.

The effects of LPS and rhTNF_
on lung lymph measurements contrast in several ways. First, LPS caused greater early increases of Ql, Clp, and PAP than did rhTNF_
. Cyclooxygenase blockade of sheep given LPS attenuates the early, acute pulmonary hypertension and the early, but not late, increases
of $Q_l$ and $C_l$. Second, whereas 1 µg/kg LPS induced briefer release of prostacyclin, no systemic vasodilation, and lower fluid transfusion requirements than did 25–150 µg/kg rhTNF, LPS caused a greater increase of lung lymph transvascular protein clearance after 6 hours. Our results suggest that differences of eicosanoid mediator profile and pulmonary hydrostatic pressure are important determinants of the early differences of $Q_l$ and $C_l$ between sheep given LPS and rhTNF. Subsequent differences of $Q_l$ and $C_l$ appear due to the greater increase of pulmonary microvascular permeability caused by LPS infusion. As a putative mediator of the lethal effects of LPS in animals, it is possible that rhTNF partly contributes to the increase of lung microvascular permeability after

**Table 5. Total Plasma Protein Concentration**

<table>
<thead>
<tr>
<th>Plasma Protein (gm/dl)</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>rhTNF (25–150 µg/kg)</td>
<td>5.4±0.2</td>
</tr>
<tr>
<td>M+rhTNF (25 µg/kg)</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>M+rhTNF (25 µg/kg)</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>rhLT (25 µg/kg)</td>
<td>4.6±0.4</td>
</tr>
<tr>
<td>LPS (1 µg/kg)</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

rhTN, recombinant human tumor necrosis factor alpha; M+rhTNF, meclofenamate (10 mg/kg)+rhTNF; 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>rhTNF (75–150 µg/kg)</td>
<td>473±18</td>
</tr>
<tr>
<td>rhTNF (25 µg/kg)</td>
<td>510±37</td>
</tr>
<tr>
<td>M+rhTNF (25 µg/kg)</td>
<td>507±12</td>
</tr>
<tr>
<td>rhLT (25 µg/kg)</td>
<td>461±44</td>
</tr>
<tr>
<td>LPS (1 µg/kg)</td>
<td>527±58</td>
</tr>
<tr>
<td>Meclofenamate alone</td>
<td>397±68</td>
</tr>
<tr>
<td>Control</td>
<td>461±41</td>
</tr>
</tbody>
</table>

Arterial pH

| rhTNF (75–150 µg/kg) | 7.45±0.06 | 7.23±0.04*† |
| rhTNF (25 µg/kg)     | 7.39±0.0 | 7.20±0.05*† |
| M+rhTNF (25 µg/kg)   | 7.48±0.03 | 7.37±0.03 |
| rhLT                 | 7.45±0.03 | 7.42±0.05 |
| LPS                  | 7.50±0.02 | 7.46±0.02 |
| Meclofenamate        | 7.35±0.03† | 7.44±0.01* |
| Control              | 7.45±0.02 | 7.43±0.04 |

<table>
<thead>
<tr>
<th>Qs/Qr (percent cardiac output)</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhTNF (75–150 µg/kg)</td>
<td>15±1</td>
</tr>
<tr>
<td>rhTNF (25 µg/kg)</td>
<td>15±3</td>
</tr>
<tr>
<td>M+rhTNF (25 µg/kg)</td>
<td>15±3</td>
</tr>
<tr>
<td>rhLT</td>
<td>18±4</td>
</tr>
<tr>
<td>LPS</td>
<td>16±4</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>19±4</td>
</tr>
<tr>
<td>Control</td>
<td>17±4</td>
</tr>
</tbody>
</table>

rhTNF, recombinant human tumor necrosis factor alpha; M+rhTNF, meclofenamate (10 mg/kg)+rhTNF; rhLT, recombinant human lymphotoxin; LPS, Escherichia coli lipopolysaccharide; Qs/Qr, lung shunt fraction as percent of right cardiac output. Results from sheep treated with rhTNF 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 rhTNF.

*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 airflow 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. 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 airflow 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 lipooxygenase products, as well as major fluid transfusion may contribute to airflow closure and atelectasis, increased shunt fraction, hypoxemia, and increased airflow 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 LPS17,18,30,33 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 vasodilatation 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-PGF₁α levels in sheep given either LPS or rhTNFα may be due to multiple factors. The delay between LPS stimulation of macrophages and subsequent TNFα release probably contributes to the one hour delay before lymph 6-k-PGF₁α levels increase significantly; however, this does not explain the decline of 6-k-PGF₁α levels in LPS-treated sheep to baseline values after 4 hours. Lung lymph 6-k-PGF₁α levels were elevated throughout the study after rhTNFα infusion. The doses of rhTNFα we infused may produce higher plasma levels of TNFα than would be produced by infusion of 1 µg/kg LPS. In addition, the functional half-lives of rhTNFα 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 µg/kg E. coli LPS, 25–150 µg/kg rhTNFα, and 25 µg/kg rhLT. Whereas both LPS and 25–150 µg/kg rhTNFα acutely caused severe leukopenia and, after 6 hours, comparably increased PAP and PVR, rhTNFα 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 rhTNFα, 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 rhTNFα. By studying doses of rhTNFα, 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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