# Leukotrienes but not Complement Mediate Limb Ischemia-Induced Lung Injury

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**Reperfusion after limb ischemia leads to sequestration of polymorphonuclear leukocytes (PMN) in the lungs and to leukocyte- (WBC) and thromboxane- (Tx) dependent respiratory dysfunction. This study examines the intermediary role of the chemoattractants leukotriene (LT)$B_4$ and complement (C) fragments. Anesthetized sheep with chronic lung lymph fistulae underwent 2 hours of tourniquet ischemia of both hind limbs. In untreated controls (n = 7), 1 minute after tourniquet release, mean pulmonary artery pressure (MPAP) rose from 13 to 38 mmHg ($p < 0.05$) and returned to baseline within 30 minutes. Pulmonary artery wedge pressure was unchanged from 3.6 mmHg. There were increases in plasma $LTB_4$ levels from 2.46 to 9.34 ng/ml ($p < 0.01$), plasma $TxB_2$ levels from 211 to 735 pg/ml ($p < 0.05$), and lung lymph $TxB_2$ from 400 to 1005 pg/ml ($p < 0.05$). C3 levels were 96% of baseline values. Thirty minutes after reperfusion, lung lymph flow ($\dot{Q}L$) increased from 4.3 to 8.3 ml/30 minutes ($p < 0.05$), lymph/plasma protein ratio was unchanged from 0.6, and the lymph protein clearance increased from 2.6 to 4.6 ml/30 minutes ($p < 0.05$), data consistent with increased microvascular permeability. WBC count fell within the first hour from 6853 to 3793/$mm^3$ ($p < 0.01$). Lung histology showed leukosequestration, 62 PMN/10 high-power fields (HPF) and proteinaceous exudates. In contrast to this untreated ischemic group, animals treated with the lypoxygenase inhibitor diethylcarbamazine (n = 5) demonstrated a blunted reperfusion-induced rise in MPAP to 17 mmHg ($p < 0.05$). There were no increases in $LTB_4$, $TxB_2$, $\dot{Q}L$ or lymph protein clearance ($p < 0.05$). WBC count was unchanged and lung leukosequestration was reduced to 40 PMN/10 HPF ($p < 0.05$). Decomplementation with cobra venom factor (n = 4) resulted in plasma C3 levels, 10% of baseline, but tourniquet release still led to pulmonary hypertension, elevated $LTB_4$, $TxB_2$ levels, and a decline in WBC count similar to that of untreated ischemic control animals. Histology showed 46 PMN/10 HPF sequestered in the lungs. Further, bilateral hind limb ischemia in either genetically sufficient (n = 10) or deficient (n = 10) C5 mice led to significant lung leukosequestration of 108 and 106 PMN/10 HPF, respectively, compared with 42 and 47 PMN/10 HPF in sham C5(+) and C5 (−) mice (n = 20) ($p < 0.01$). These results suggest that the lung leukosequestration and increased microvascular permeability after lower torso ischemia are mediated by the chemotactic agent $LTB_4$, but not by the complement system.**

LOWER TORSO ISCHEMIA followed by reperfusion leads to respiratory failure associated with sequestration of neutrophils in the lungs.[1,2] The accumulation and activation of these inflammatory cells is believed to be caused by chemotactic agents generated by the ischemic tissue and released into the circulation. Thus, leukotriene (LT) $B_4$ as well as the complement fragments C5 and C5a, when infused intravenously, have been shown to lead to thromboxane (Tx) generation, lung leukosequestration and injury.[3-5] The role of these agents in ischemic injury is suggested by reports that myocardial damage after experimental coronary occlusion can be significantly reduced by LT inhibition[6] or by inactivation of the complement system.[7] Other evidence that points to the role of chemoattractants is less direct. For example, intravascular thrombin activation and associated lung microembolization, events considered by some[8] to be central to lung injury after lower torso ischemia, are complement-dependent.[9] This study was designed to test directly the role of the chemoattractants, $LTB_4$, and the complement system in mediating limb ischemia-induced lung injury.

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## Methods

### *Sheep Preparation*

Female sheep (n = 19) weighing 25–44 kg underwent cannulation of the lung lymphatic according to a modification of the technique described by Staub.[10] Animals were anesthetized with intravenous (I.V.) pentobarbital sodium 15 mg/kg, paralyzed with 2 mg of pancuronium bromide, intubated, and mechanically ventilated with a volume cycled respirator using room air. Through a right posterolateral thoracotomy, the efferent duct of the caudal mediastinal lymph node was cannulated with a heparinized silastic catheter (No. 602-155, Dow Corning Corp., Midland, MI). The distal portion of the lymph node, just below the level of the inferior pulmonary ligament, was transected and ligated, and the diaphragm around the lymph node circumferentially cauterized. Systemic lymph tributaries to the proximal portion of the lymph node were cauterized or ligated to minimize extra-pulmonary contamination of collected lymph. The thoracotomy was closed and the lymphatic cannula was exteriorized through the chest wall. A thermistor-tipped pulmonary arterial (Electro-cath Corp, Rahway, NJ) and a central venous catheter were introduced through the right internal jugular vein. The aorta was cannulated via the adjacent carotid artery. After a recovery period of 4 to 5 days, when animals appeared vigorous, were afebrile, and had a steady flow of blood free lymph, the experiment was conducted.

### *Cardiopulmonary Function*

Strain-gauge transducers (Model D-240, Bently Laboratories, Inc., Irvine, CA) were used to measure the following pressures: mean arterial (MAP), mean pulmonary arterial (MPAP), and pulmonary arterial wedge (PAWP). The pulmonary microvascular pressure (Pmv) was calculated from the Gaar equation, Pmv = PAWP + 0.4 (MPAP - PAWP).[11] Pulse rate was determined from an arterial pressure trace. Cardiac output (CO) was measured in triplicate by thermodilation (Model 5000, Electro-Cath Corp., Rahway, NJ). Blood gases, pH, oxygen saturation, and hemoglobin were measured with Clark and Severinghaus electrodes and by spectrophotometry using extinction coefficient specific to sheep blood (Model 813 and 282, Instrumentation Laboratory, Lexington, MA).

### *Hematology*

Circulating platelets and white blood cells (WBC) were counted by means of phase microscopy.

### *Biochemical Assays*

Plasma and lymph concentrations of $TxB_2$ and 6-keto-PGF1$\alpha$, the stable hydrolysis products of TxA2 and prostacyclin, were measured in duplicate by radioimmunoassay.[12] Blood was drawn into cooled syringes containing ethylene diamine tetracetic acid (EDTA) and aspirin. The blood was immediately centrifuged at 1500 × g at 4 C for 20 minutes, and the plasma was separated and stored at −20 C until assayed.

Concentrations of $LTB_4$ in plasma and lung lymph were measured in duplicate by radioimmunoassay[13] using a rabbit antibody and standards supplied by Seragen (Cambridge, MA). Cross reactivity of anti-$LTB_4$ antibody with other LTs, $TxB_2$, the prostaglandins (PGs), and their metabolites was less than 1%. Serum levels of the third component of complement (C3) were quantified immunochemically using a rate nephelometer (Immuno-chemistry Analyzer, Auto ICS, Becham Instruments, Brea, AC) and rabbit anti-sheep C3 antibody (Cappel Lab., West Chester, PA). Because complement standards for the serum of sheep are not commercially available, a pool of ten sera obtained from healthy sheep was used as our reference. The value obtained was assigned a level of 100% C3. The measured C3 levels are expressed as a percentage of this normal serum pool.

Lung lymph was collected at 15- or 30-minute intervals in cold, graduated test tubes containing EDTA and aspirin. The lymph was then centrifuged at 1500 × g and 4 C for 20 minutes, and the supernatant was separated and stored at −20 C until assayed for TxB2, 6-keto-PGF1$\alpha$, and $LTB_4$. Lymph and plasma total protein concentrations were determined in duplicate by the spectrophotometric protein dye method described by Bradford.[14]

### *Histologic Examinations*

At the end of the experiment, animals were euthanized with an overdose of pentobarbital and potassium chloride. Glutaraldehyde (2.5%) was instilled into the lungs through an endotracheal tube at a pressure of 25 cm of $H_2O$. After 20 minutes. the hilum of the left lung was clamped. The lung was removed and immersed in glutaraldehyde for 72 hours. Tissue samples were then taken in a uniform manner from identical, dependent lung regions. All microscopic sections were stained with hematoxylin and eosin and were interpreted by a pulmonary pathologist (Lester Kobzik) in a blinded fashion. Lung sequestration of neutrophils was quantitated by counting alveolar septal wall neutrophils in ten randomly chosen high-powered fields (1000x). Microscopic fields in proximity to bronchial structures, pleura, and large vessels were excluded.

### *Experimental Protocol—Sheep*

Experiments were conducted in anesthetized sheep, placed supine, ventilated at a tidal volume of 15 ml/kg and a rate of 12–15 cycles/minute, adjusted to keep the $PaCO_2$ levels between 30 and 35 mmHg. The state of

anesthesia was maintained with a continuous infusion of 0.1 mg/kg/minute of pentobarbital, and 30 μg/kg/minute of pancuronium. Saline, in an infusion of 7 ml/kg/hour, was infused throughout the course of the experiment. External heat was used to maintain body temperature between 38 C and 39 C. After 2 hours of stabilization, baseline measurements were taken. Both hind-limbs were elevated for 2 minutes to drain them of blood, and tourniquets were applied as high on the thighs as possible and inflated to 300 mmHg. After 2 hours of ischemia, the tourniquets were removed and the animals were monitored for another 2 hours.

Studies were conducted in untreated ischemic animals (n = 7) or in sheep pretreated with the lypoxygenase inhibitor diethylcarbamazine[15] (DEC, Sigma, St. Louis, MO) (n = 5). Because we speculated that $LTB_4$ might be released from the ischemic tissue, DEC was started 30 minutes before tourniquet application to achieve an adequate concentration in the ischemic limb. DEC was infused intravenously at 90 mg/kg over 20 minutes, followed by a constant infusion of 0.9 mg/kg/min. This dosage was based on the thesis that if DEC is distributed uniformly in body water, a lowest concentration of 150 mg/L or 380 μM would result. *In vitro* studies showed that 90% inhibition of $LTB_4$ synthesis was achieved at 250 uM DEC.[15]

In a third study, sheep (n=4) were depleted of complement by repeated I.V. injections of purified cobra venom factor (CVF)[16] isolated from the snake Naje haje (Cordis Lab, Miami, FL), 10 U/kg. The CVF was given three times per day for 3 days. The last injection was given 18 hours before the experiment. Sham sheep (n = 3) were given 6 hours of anesthesia without hind-limb ischemia.

*Mice Studies*

Because depletion of complement with CVF is not complete and may not affect C5 levels in lung parenchyma or ischemic tissues, and because CVF itself leads to lung leukosequestration[17] complicating the evaluation of this end point, additional studies were performed in complement-deficient mice. Two congenic strains were used, B10D2/Nsn C5-sufficient mice (C5+) (n = 20) and B10D2/Osn C5-deficient mice (C5−) (n = 20) (Jackson Lab, Bar Harbor, ME). All studies were conducted using males because of reports of intermediate C5 levels in females of the deficient strain.[18] The mice, weighing between 20 and 30 g, were anesthetized with 60 mg/kg of intraperitoneal pentobarbital sodium. C5+ (n = 10) and C5− (n = 10) mice were subjected to bilateral hind-limb ischemia using rubber bands. These bands were applied after a 2-minute period of hind-limb elevation to minimize retained blood. The animals were then placed in the prone position, and after 3 hours, the tourniquets were removed. After another 3 hours of reperfusion, the mice were killed with an intraperitoneal overdose of sodium pentobarbital, and the lungs were harvested using the following technique. Through a tracheostomy, 2.5% glutaraldehyde was introduced at a pressure of 20 cm of $H_2O$ for 20 minutes. The trachea was then ligated, and the lungs were removed and bathed in glutaraldehyde for 24 hours. Sections for histology were taken from identical dependent lung regions and stained with hematoxylin and eosin. Quantitation of PMN was done as in sheep lungs. The lungs of sham controls C5+ (n = 10) and C5− (n = 10) mice were also studied. In these animals, all procedures were done as in the experimental group, except that the hind-limbs were not made ischemic.

Results are expressed in the text and figures as mean ± standard error. Differences between means were tested by an analysis of variance, paired, and nonpaired t-test. When multiple comparisons were made, the Bonferroni procedure was applied.[19] Significance was accepted if $p < 0.05$.

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. 78-23, revised 1978).

## Results

During the 2 hours of tourniquet ischemia, there were no alterations in cardiopulmonary function, blood counts, or prostanoid levels. One minute after tourniquet release, MPAP rose from 13 ± 1 to 38 ± 4 mmHg ($p < 0.05$) and returned to baseline levels within 30 minutes (Fig. 1). There was a similar transient rise in Pmv from 7 ± 1 to 18 ± 2 mmHg ($p < 0.05$), whereas the PAWP was unchanged from 4 ± 1 mmHg. Also unchanged were blood gases, systemic arterial pressure, and CO.

Lung $\dot{Q}L$ increased from a baseline value of 4.3 ± 0.6 to 8.3 ± 1.8 ml/30 minutes ($p < 0.05$) and remained elevated for 2 hours (Fig. 2). The lymph/plasma (L/P) protein ratio was unchanged from 0.60 ± 0.03, and the lymph protein clearance increased from 2.6 ± 0.4 to 4.6 ± 0.8 ml/30 minutes ($p < 0.05$) (Fig. 2). Plasma $LTB_4$ levels rose from 2.5 ± 0.6 to 9.3 ± 2.4 ng/ml ($p < 0.01$) at 2 minutes of reperfusion and returned to baseline levels at 30 minutes (Fig. 3). Lung lymph $LTB_4$ concentrations were unchanged during reperfusion from baseline values of 0.7 ± 0.1 ng/ml. Levels of the complement fragment C3 were also unchanged (97% of baseline levels that were 96% of C3 levels in pooled sheep sera) during ischemia and reperfusion.

Plasma TxB2 levels rose from 211 ± 21 to 735 ± 112 pg/ml ($p < 0.05$) after 2 minutes of reperfusion and re-

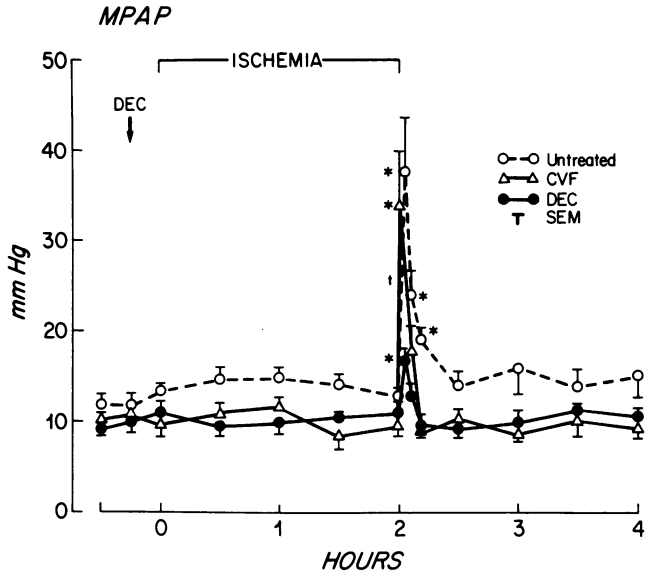



FIG. 1. Tourniquet release after 2 hours of bilateral hind-limb ischemia led to an immediate rise in MPAP. The pulmonary hypertension was attenuated by pretreatment with the lypoxygenase inhibitor DEC but not by decomplementation using CVF.
*Significant differences relative to baseline values.
†Significant differences between untreated animals and the DEC group.

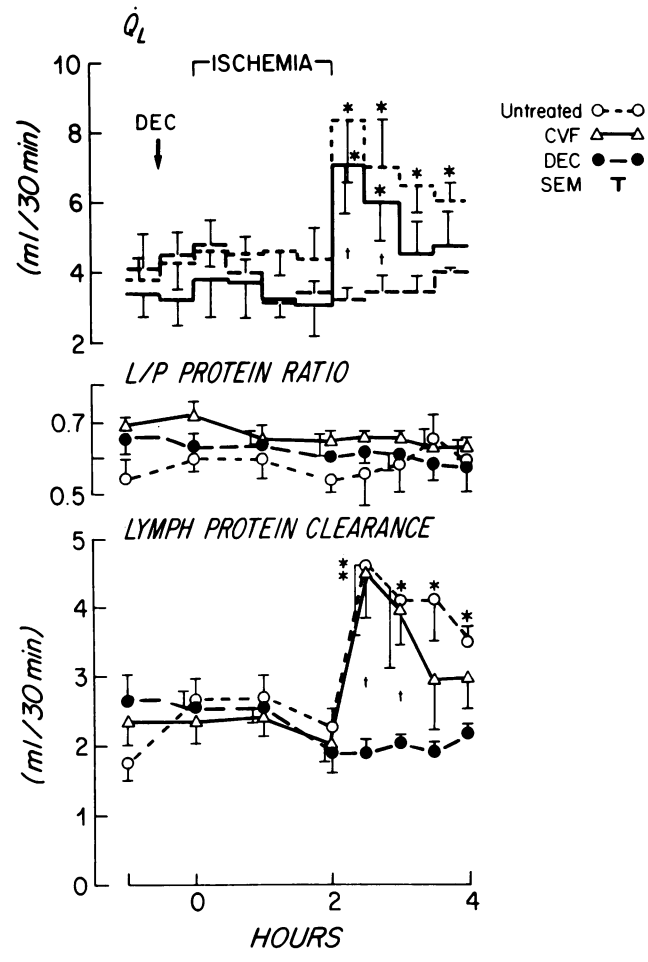



FIG. 2. During reperfusion lung lymph flow, ($\dot{Q}L$) rose, the L/P protein ratio was unchanged, and lymph protein clearance increased. These changes are consistent with increased lung microvascular permeability. DEC prevented the rises in $\dot{Q}L$ and lymph protein clearance, whereas CVF did not provide protection.
*Significance relative to baseline.
†Significance when untreated animals and DEC animals are compared.

turned to baseline levels at 30 minutes (Fig. 4). Lung lymph TxB2 levels rose from 406 ± 41 to 1005 ± 530 pg/ml ($p < 0.05$) and remained elevated above baseline ($p < 0.05$) for a more prolonged period than plasma levels (Fig. 4). Plasma and lymph 6-keto-$PGF_{1\alpha}$ concentrations were unchanged during ischemia and reperfusion from baseline values of 15 ± 8 and 89 ± 54 pg/ml, respectively.

The circulating WBC count fell during reperfusion in each saline-treated sheep. The average decline during the first hour was from 6853 ± 1149 to 3796 ± 874 /$mm^3$ ($p < 0.01$). After the second hour, the WBC count had returned to 6583 ± 1749/$mm^3$.

Platelet counts were unchanged by ischemia and reperfusion from their baseline values of 305,000 ± 106,000/$mm^3$.

Histologic examination of the lungs revealed accumulations of PMN within alveolar capillaries throughout the lung parenchyma (Figs. 5A–D), along with some foci of proteinaceous exudate within alveolar spaces. Neither platelet nor thrombin microaggregates were observed. Quantitative PMN counts showed 62 ± 3 PMN/10HPF, in contrast to 22 ± 3 PMN/10 HPF in lungs of sham animals.

Infusion of DEC caused a transient decrease in MAP from 91 ± 4 to 69 ± 7 mmHg ($p < 0.05$) and CO from 3.34 to 2.89 L/min ($p < 0.05$). Both variables returned to baseline values within 15 minutes. There were no changes in MPAP, Pmv, PAWP, $\dot{Q}L$, L/P, WBC, or platelet counts (Figs. 1 and 2). Further, plasma and lung lymph

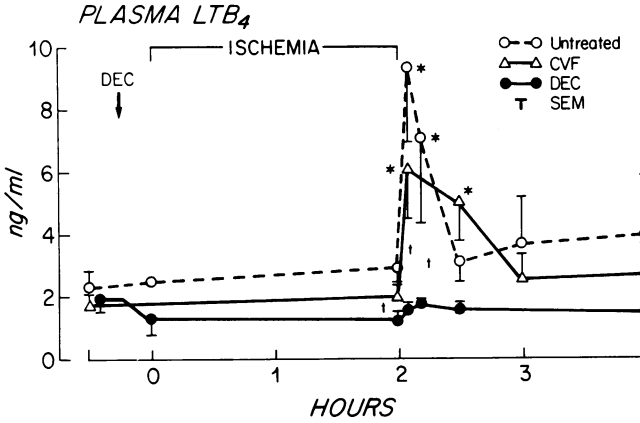



FIG. 3. Reperfusion led to a transient increase in plasma leukotriene (LT)$B_4$ levels. Pretreatment with DEC decreased baseline $LTB_4$ levels and prevented the reperfusion-induced generation of $LTB_4$. Decomplementation (CVF) did not affect $LTB_4$ levels relative to untreated animals.
*Significance relative to baseline.
†Significance between untreated and DEC groups.

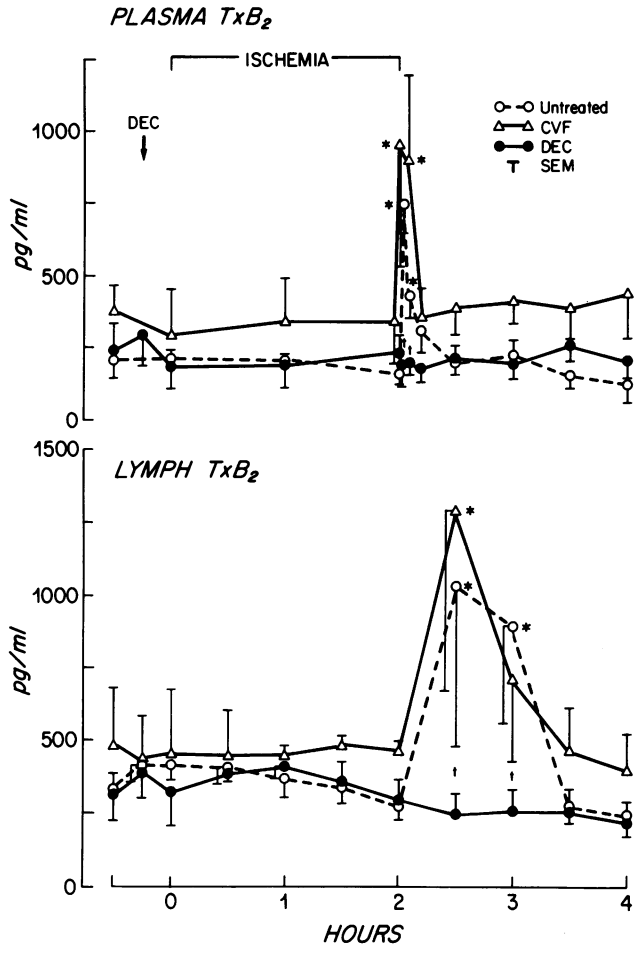



FIG. 4. Tourniquet release led to an increase in plasma $TxB_2$ levels. A higher and more sustained rise of $TxB_2$ concentration occurred in lung lymph. DEC prevented the reperfusion-induced synthesis of $TxB_2$, whereas CVF led to changes similar to those of untreated animals.
*Significance relative to baseline.
†Significance between untreated and DEC groups.

TxB2 and 6-keto-$PGF_{1\alpha}$ levels were unchanged (Fig. 4). Plasma $LTB_4$ levels decreased from 2.9 ± 0.2 to 1.5 ± 0.2 ng/ml ($p < 0.05$) 30 minutes after DEC infusion (Fig. 2). After tourniquet release, there was an increase in MPAP from 11.1 ± 1 to 16.7 ± 1.1 mmHg ($p < 0.05$), but this value was lower than that seen in untreated ischemic control animals ($p < 0.05$) (Fig. 1). In addition, Pmv rose from 6 ± 1 to 9 ± 1 mmHg ($p < 0.05$) with an unchanged PAWP of 3 ± 1 mmHg. DEC prevented reperfusion-induced increases in $\dot{Q}L$, lymph protein clearance, as well as in the elevations in plasma $LTB_4$ and plasma and lymph TxB2 levels ($p < 0.05$, respectively) (Figs. 2–4). The circulating WBC counts during reperfusion were unchanged from 6526 ± 1343/$mm^3$. Pulmonary parenchymal neutrophil counts were 40 ± 3 PMN/10 HPF lower than those of untreated ischemic sheep (Figs. 5A–D).

Treatment with CVF reduced plasma C3 levels to 10.8 ± 0.8% ($p < 0.05$) of pretreatment levels (102% of C3 levels in pooled sheep sera). Baseline cardiopulmonary function, eicosanoids levels, and WBC counts were unchanged relative to untreated ischemic and sham groups. Tourniquet release in these complement-depleted sheep led to increases in MPAP, Pmv, $\dot{Q}L$, lymph protein clearance, plasma $LTB_4$ concentration, and TxB2 levels in plasma and lung lymph similar to values in untreated ischemic animals (Figs. 1–4). Further, WBC counts fell during reperfusion to 2100 ± 600/$mm^3$ ($p < 0.05$), and lung histology showed moderate leukosequestration of 46 ± 6 PMN/10 HPF (Figs. 5A–D).

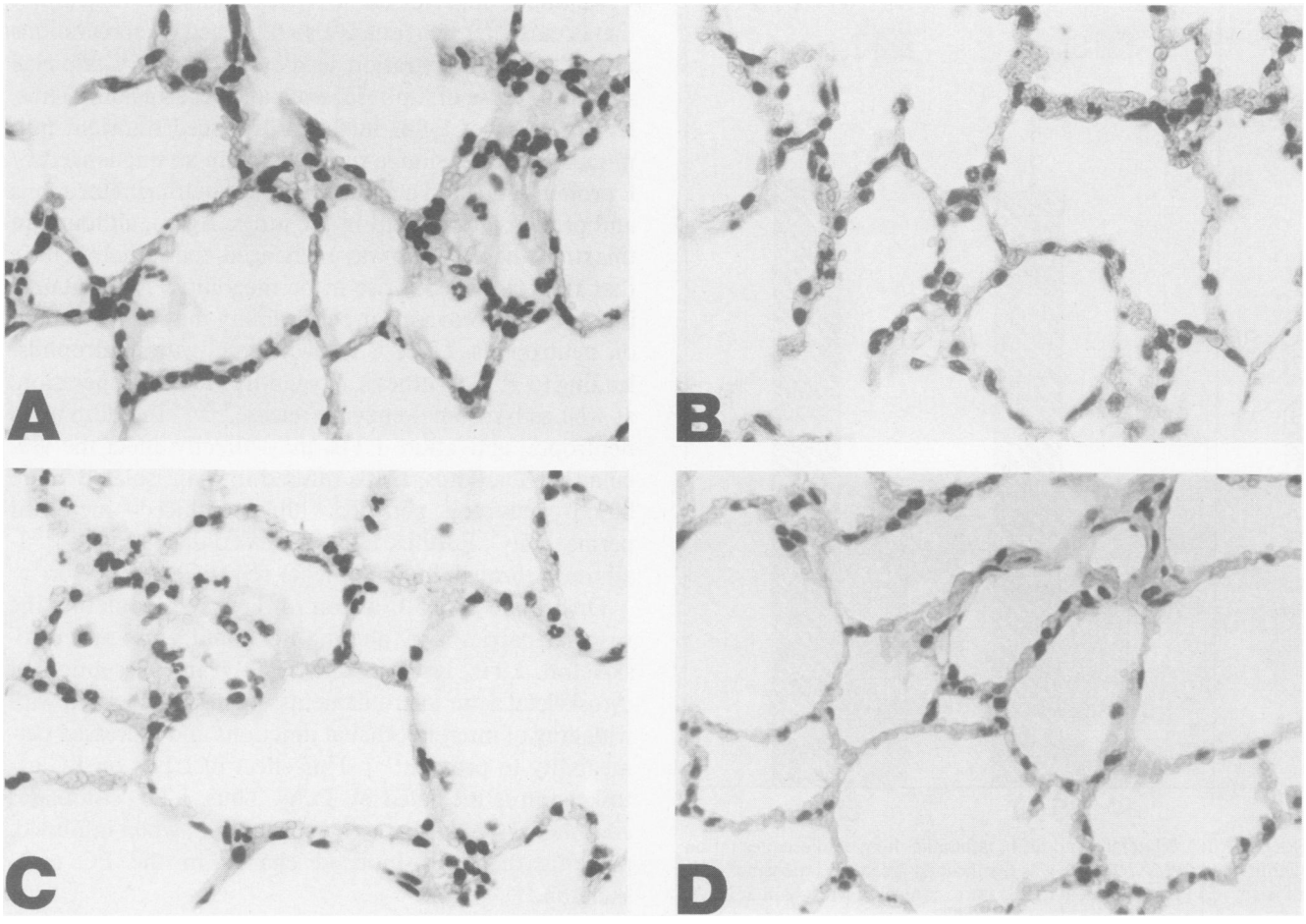
Bilateral hind limb ischemia and reperfusion in both genetically sufficient or deficient C5 mice led to similar lung leukosequestration of 108 ± 10 and 106 ± 16 PMN/10 HPF, respectively. These values were higher than those seen in sham C5(+) mice of 42 ± 9 PMN/10 HPF ($p < 0.01$) and than those seen in sham C5(−) mice of 47 ± 5 PMN/10 HPF ($p < 0.01$) (Figs. 6 and 7).

## Discussion

Two hours of lower torso ischemia followed by reperfusion led to lung injury manifest by pulmonary hypertension and increased microvascular permeability. The lymph data used to assess permeability—that is, the rise in $\dot{Q}L$ and protein clearance—are consistent with increased permeability but do not prove it. It is possible that the increase in $\dot{Q}L$ with an unchanged L/P protein ratio were due to an increase in pulmonary vascular surface area rather than to an altered permeability. This possibility was excluded in previous studies of limb ischemia, where lung vascular surface area was fully recruited by inflation of a left atrial balloon. In this setting of a pressure independent L/P protein ratio, reperfusion leads to a rise in both $\dot{Q}L$ and the L/P ratio and a decrease in the osmotic reflection coefficient, documenting increased lung microvascular permeability.[20]

Previous studies have demonstrated that tissue ischemia-mediated lung injury requires generation of TxA2 and the presence of WBC.[1,2] Thus, in animals pretreated with a Tx synthetase inhibitor or rendered leukopenic, lung injury is prevented.[20,21] The major site of Tx synthesis after tourniquet release appears to be the lungs. This is indicated by the high and prolonged elevation of $TxB_2$ concentration in lung lymph as well as the transpulmonary concentration gradient of $TxB_2$, where aortic $TxB_2$ levels are higher than those in pulmonary artery blood.[22] The source of $TxA_2$ may be from sequestered PMN or from lung parenchyma.[23]

We postulate that tissue ischemia stimulates the local generation of a chemoattractant, which is released into the circulation and activates neutrophils to be sequestered in the lungs. The observation that plasma $LTB_4$ levels rise after ischemia suggests the possibility that this agent is




FIGS. 5A–D. (A) Neutrophil sequestration was found in alveolar capillaries after 2 hours of limb ischemia. (B) Pretreatment with DEC reduced the pulmonary leukosequestration, whereas (C) CVF led to lung neutrophil accumulations. (D) The lung of sham animals appears normal (original magnification ×600).

the mediator. The relatively high baseline $LTB_4$ levels obtained by radioimmunoassay in plasma and lymph may be an error and may far exceed their true concentration.[24] Thus, WBC contained in the blood may be activated to secrete $LTB_4$ by the withdrawal and manipulation process that takes place before assay. It is also possible that the $LTB_4$ antibody cross-reacted with unknown plasma lipids or proteins. Nevertheless, we believe that the fourfold increase in plasma $LTB_4$ concentration during reperfusion reflects the true relative magnitude of change in endogenous $LTB_4$ synthesis.

There are a number of potential $LTB_4$ synthetic sites, particularly mast cells as well as WBCs contained in tissue and blood vessels of the ischemic limb.[4,6,24] In the ischemic limb, neutrophils are found adherent to capillary endothelium.[25] This has been attributed to diminished flow and to rheologic properties of WBC. It is possible that these adherent WBC are activated to synthesize $LTB_4$. An alternate explanation for PMN adherence in the ischemic tissue is that the neutrophils are responding to tissue chemoattractants such as $LTB_4$. If the latter is true, then tissue macrophages and mast cells must be an important $LTB_4$ source. Further, the recent observation that endothelial cells can generate $LTB_4$ makes their contribution possible.[26] The lungs themselves are unlikely to be major contributors of $LTB_4$ synthesis, given the observation that $LTB_4$ levels in lung lymph were not elevated during reperfusion. These considerations indicate separate synthetic sites for $LTB_4$ and $TxA_2$, the lungs being prominent with regard to the latter eicosanoid.

The central role of $LTB_4$ in mediating limb ischemia-induced lung injury is suggested by the observation that inhibiting its synthesis through the use of DEC prevented the lung injury. However, this protection does not unequivocally prove a role for $LTB_4$. The possibility that DEC induced its effect through another pathway unrelated to lypoxygenase inhibition, such as antioxidant activity, cannot be excluded. Further, because DEC also inhibits the synthesis of the peptidoleukotrienes $C_4$ and $D_4$,[15] involvement of these agents is also possible. Both $LTC_4$ and

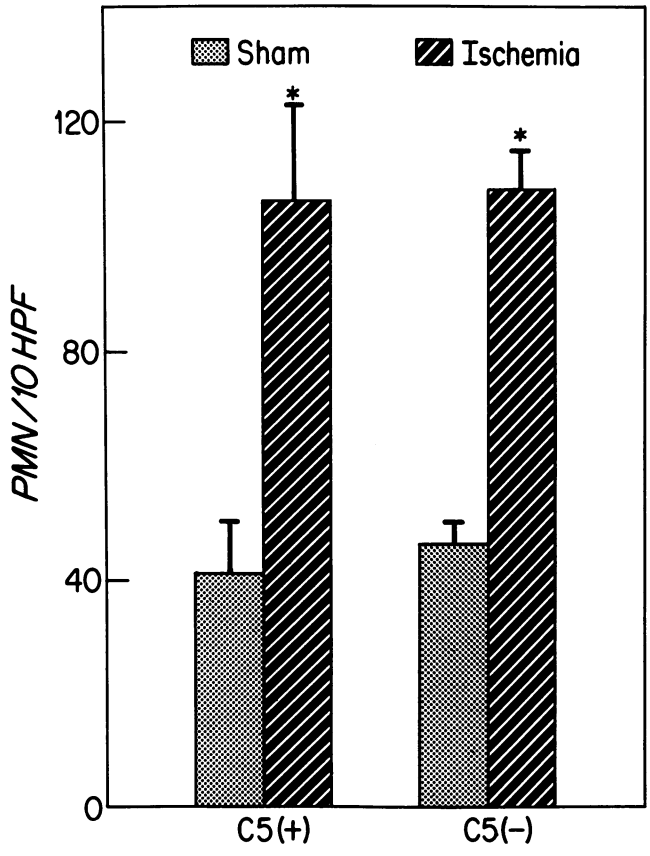



FIG. 6. After bilateral hind-limb ischemia, lung leukosequestration quantitated as PMN/10 HPF was significantly greater in both genetically sufficient (C5+) and deficient (C5−) mice, compared with sham animals. *Significance relative to sham animals.

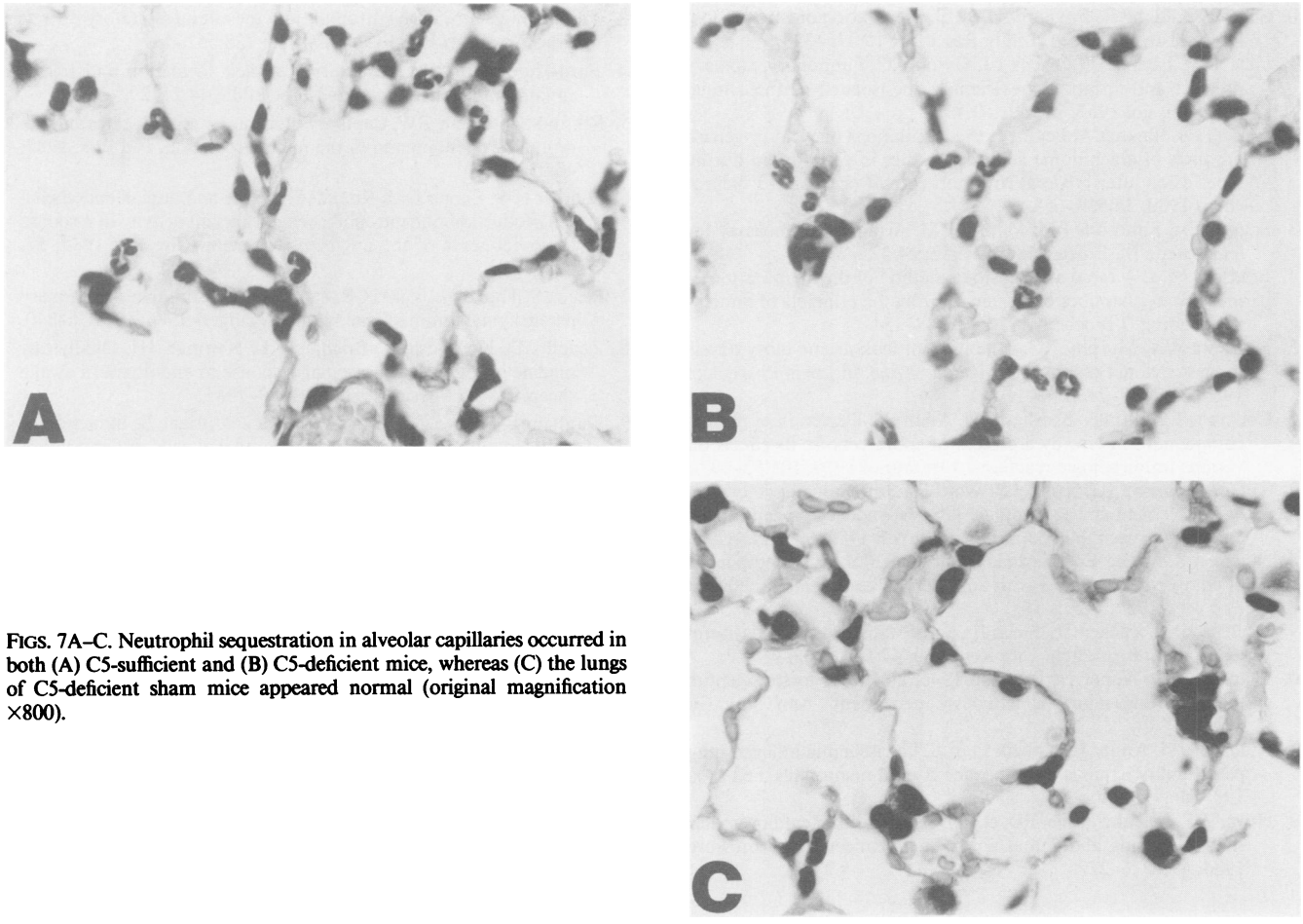
$LTD_4$ can induce pulmonary vasoconstriction, Tx generation, and possibly increased microvascular permeability.[4,6,24] However, $LTC_4$ and $LTD_4$ are not chemoattractants and thus are unlikely mediators of lung WBC entrapment. Further, it is unlikely that DEC provides protection through redirection of arachidonic acid to the cyclooxygenase pathway leading to increased synthesis of vasodilating prostaglandins, because 6-keto-$PGF_{1a}$ levels were unaffected by DEC. Finally, the possibility that DEC exerts at least part of its effect by Tx inhibition must be considered. There is no evidence that DEC is a primary inhibitor of Tx, because DEC does not lower blood Tx levels in other experimental settings.[27,28] However, DEC does prevent the rise in $TxB_2$ after ischemia. This is most likely an indirect action. Thus, $LTB_4$ can stimulate tissue such as endothelium[29] and PMN[30] to synthesize Tx. DEC, by limiting $LTB_4$ synthesis, may therefore limit the rise in $TxB_2$. It is likely that this secondary LT-induced generation of $TxA_2$ is of great importance. Thus, in other studies, $LTB_4$, when applied to abraided skin or injected subcutaneously, led to local PMN accumulations and Tx generation, events that were prevented with a Tx synthetase inhibitor.[3]

An important role of $LTB_4$ in mediating the lung injury that occurs after ischemia is also suggested by observations that $LTB_4$ administration leads to pulmonary sequelae similar to those of limb ischemia and reperfusion.[4] Thus, I.V. infusion of $LTB_4$ in sheep produced transient pulmonary hypertension, a rise in $\dot{Q}L$ with an unchanged L/P protein ratio, and a reduction of circulating neutrophils and of their entrapment in the lungs. Again, although in this study the L/P ratio was unchanged, the authors believe that $LTB_4$ induced a rise in permeability. This putative increase in microvascular permeability may be dependent on neutrophils. $LTB_4$ is known to activate neutrophils, leading to $TxA_2$ synthesis, oxygen-free radical generation, as well as lysosomal enzyme release.[6,23,24] In addition to neutrophil activation, $LTB_4$ may directly affect the vascular barrier. Thus, $LTB_4$ infused into an isolated white blood cell-free, perfused lung, induced increased permeability[4]. Further, $LTB_4$ increased the passage of $^{125}$I-albumin through an endothelial cell (EC) monolayer.[4]

One mode of $LTB_4$ action in directly modulating the vascular barrier is by altering EC architecture and cytoskeleton. $LTB_4$ leads to disassembly and disruption of cytoskeletal actin microfilaments.[31] This is associated with widening of interendothelial junctions and increased permeability to protein.[21,32] This effect of $LTB_4$ on EC cytoskeleton is mediated by $TxA_2$. Thus, $LTB_4$ stimulates endothelial Tx synthesis,[29] an event that, when inhibited, prevents the $LTB_4$-induced change in the EC cytoskeleton.[32]

Although $LTB_4$ appears to account for the lung leukosequestration and injury that occur after limb ischemia, other chemotactic agents may also be involved. Thus oxygen-free radicals (OFR) have been implicated in ischemia-reperfusion injury.[33] These agents may produce direct damage or may induce the generation of chemoattractants that are secondarily injurious. Because OFR activate the arachidonic acid cascade, it is plausible that they may serve as a stimulus for the synthesis of $LTB_4$ and $TxA_2$.[34]

The observation that C3 levels were unchanged during ischemia and reperfusion does not necessarily indicate that the complement system was not activated and consumed, because the assay for C3 using polyclonal antibodies may measure complement degradation as well as precursor products. However, other evidence indicates that the chemotactic proteins of the complement system do not play a role in ischemia-induced lung injury. Thus, depletion of C3 by CVF, and thereby reduction of levels of the terminal components C5 to C9, is not protective, and lung leukosequestration is not prevented in genetically deficient C5 animals. That C5 is unlikely to mediate the lung injury after ischemia is further suggested by reports that, unlike ischemia-induced leukosequestration,[1,2,21] complement-induced lung leukosequestration is not Tx-




FIGS. 7A–C. Neutrophil sequestration in alveolar capillaries occurred in both (A) C5-sufficient and (B) C5-deficient mice, whereas (C) the lungs of C5-deficient sham mice appeared normal (original magnification ×800).

dependent.[5] However, C5 may still play a local role in mediating WBC recruitment and injury in ischemic tissue such as that in the heart after myocardial ischemia.[7] The inability of C5 to mediate remote injury in the lungs after lower torso ischemia may be due to insufficient release of activated complement fragments into the circulation. Finally, although platelet activating factor is a strong chemoattractant, the inability of its antagonist SRI 63-072 to prevent lung leukosequestration and Tx release after limb ischemia would seem to exclude it as a mediator.[1]

In summary, the linkage between local ischemia and distant inflammation is believed to occur by synthesis of $LTB_4$ in the ischemic region and its release into the circulation during reperfusion. This event, coupled with $TxA_2$ synthesis, leads to neutrophil sequestration in the lungs with resultant increased microvascular permeability.

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