Lower Torso Ischemia-Induced Lung Injury Is Leukocyte Dependent

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Lower torso ischemia leads on reperfusion to sequestration of polymorphonuclear leukocytes (PMN) in the lungs and increased permeability. This study tests the role of circulating leukocytes (WBC) in mediating this lung injury. Anesthetized sheep prepared with chronic lung lymph fistulae underwent 2 hours of bilateral hind limb tourniquet ischemia. In untreated controls (n = 7), 1 minute after reperfusion there were transient increases in mean pulmonary arterial pressure (MPAP) from 13 to 38 mmHg ($p < 0.05$) and pulmonary microvascular pressure (Pmv) from 7 to 18 mmHg ($p < 0.05$), changes temporally related to a rise in plasma thromboxane (Tx) B_2 levels from 211 to 735 pg/ml ($p < 0.05$). Lung lymph TxB_2 levels rose from 400 to 1005 pg/ml at 30 minutes ($p < 0.05$) and remained elevated longer than plasma levels. Lung lymph flow ($\dot{Q}L$) rose from 4.3 to 8.3 ml/30 minutes ($p < 0.05$) after 30 minutes of reperfusion and remained elevated for 2 hours. The lymph/plasma (L/P) protein ratio was unchanged from 0.6, while the lymph protein clearance increased from 2.6 to 4.6 ml/30 minutes ($p < 0.05$), suggesting increased microvascular permeability. WBC counts decreased within the first hour of reperfusion from 6853 to 3796/mm^3 ($p < 0.05$), and lung histology after 2 hours showed proteinaceous exudates and leukosequestration of 62 PMN/10 high-powered fields (HPF), higher than the 22 PMN/10 HPF ($p < 0.05$) in sham animals (n = 3). Recruitment of the pulmonary vasculature by left atrial balloon inflation (n = 3) resulted in a rise in MPAP to 20 mmHg. After 3 hours of balloon inflation, $\dot{Q}L$ stabilized at 9.8 ml/15 minutes, and a pressure-independent L/P protein ratio of 0.3 was achieved. During reperfusion, $\dot{Q}L$ increased further to 11.2 ml/15 minutes, the L/P ratio rose to 0.56 and the calculated osmotic reflection coefficient decreased from 0.70 to 0.44, documenting an increase in lung microvascular permeability. In contrast to these untreated ischemic controls, sheep (n = 7) rendered leukopenic with hydroxyurea or nitrogen mustard and having a total WBC count of 760/mm^3 and PMN count of 150/mm^3 did not manifest reperfusion-induced increases in MPAP, Pmv, $\dot{Q}L$, lymph protein clearance, or lung lymph TxB_2 levels ($p < 0.05$). Plasma TxB_2 levels rose slightly at 30 minutes from 199 to 288 pg/ml ($p < 0.05$). Lung histology was normal. These data indicate that WBC mediate the ischemia-induced increase in pulmonary microvascular permeability.

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A REMOTE CONSEQUENCE of lower torso ischemia is the accumulation of neutrophils (PMN) in the pulmonary capillary bed.[1] These inflammatory cells are capable of releasing several vasotoxic agents that lead to tissue injury.[2] Such injury is noted in the lungs after PMN sequestration induced by bacteremia, endotoxemia, complement activation, or hyperoxia.[2] That circulating leukocytes (WBC) have a role in mediating increased lung permeability after lower torso ischemia is suggested by the observation that the injury can be prevented by pretreatment with the lypoxygenase inhibitor diethylcarbamazine (DEC), since this agent also prevents lung leukosequestration.[1,3] However, interpretation of these observations is clouded by the possibility that the protection provided by DEC could be due to its inhibition of leukotriene synthesis. These agents might be of primary importance in inducing lung injury and the neutrophils secondarily recruited.[4]

Pulmonary leukostasis itself is not necessarily an indication of lung injury.[5,6] In addition, several studies document that bacteremic lung injury can occur in leukopenic patients and experimental animals.[7] This study was designed to test the role of circulating leukocytes in mediating the lung injury after lower torso ischemia.

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Methods

Female sheep (n = 20) weighing 24–42 Kg underwent cannulation of the lung lymphatic according to a modification of the technique described by Staub.[8] Animals were anesthetized with intravenous (I.V.) pentobarbital sodium 15 mg/Kg, paralyzed with 2 mg pancuronium bromide, intubated, and mechanically ventilated with a mechanical 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. The diaphragm around the lymph node was circumferentially cauterized. In addition, systemic lymph tributaries to the proximal portion of the lymph node were cauterized or ligated to minimize extrapulmonary contamination of collected lymph.

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. Chloramphenicol (Parke-Davis, Morris Plains, NJ) 1 g, and gentamicin (Elkins-Sinn, Cherry Hill, NJ) 40 mg were administered IV twice daily. After a recovery period of 4–5 days when animals appeared vigorous, were afebrile, and had a steady flow of blood-free lymph, the experiment was conducted. In three of these sheep, 7 days after lung lymph cannulation, a left atrial balloon was inserted via a thoracotomy through the left fifth intercostal space. The inflated balloon had a capacity of 30 ml and was attached to a 16 French silicone elastometer-coated Foley catheter (No. S616, AM Pharmasel Co., Valencia, CA). This catheter was placed in the left atrium along with a smaller cannula to monitor pressure. Catheters were exteriorized through the chest wall and sewn to the skin. Experiments in these sheep were conducted after an additional 4–6-day period of recovery.

Stain-gauge transducers (model D-240, Bently Laboratories, Inc., Irvine, CA) were used to measure the following pressures: mean arterial (MAP), mean pulmonary arterial (MPAP), pulmonary arterial wedge (PAWP), and mean left atrial (LAP). The pulmonary microvascular pressure (Pmv) was calculated from the Gaar equation, $Pmv = PAWP + 0.4\ (MPAP - PAWP)$.[9] Pulse rate was determined from an arterial pressure trace. Cardiac output 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 with extinction coefficients specific to sheep blood (Model 813 and 282, Instrumentation Laboratory, Lexington, MA).

Circulating platelets and white blood cells (WBC) were counted by means of phase microscopy. Differential counts were made on Wright's-stained blood smears. Plasma and lymph concentrations of thromboxane (Tx) B_2 and 6-keto-prostaglandin (PG)$F_{1\alpha}$, the stable hydrolysis products of TxA_2 and prostacyclin, were measured in duplicate by radioimmunoassay.[10] 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.

Lung lymph was collected at 15- or 30-minute intervals in cold graduated tubes containing EDTA and aspirin. The lymph was then centrifuged at 1500 × *g* and 4 C for 20 minutes and the supernatant separated and stored at −20 C until assayed for TxB_2 and 6-keto-$PGF_{1\alpha}$. Lymph (L) and plasma (P) total protein concentrations were determined in duplicate by the spectophotometric protein dye method described by Bradford.[11] The L/P protein ratio was calculated and multiplied by lymph flow ($\dot{Q}L$) to obtain the lymph protein clearance. The osmotic reflection coefficient (δd) for total protein was calculated using the minimum L/P protein ratio, achieved at steady state during balloon inflation when the LAP was increased and the $\dot{Q}L$ was high.[12] At this point, the L/P protein ratio becomes independent of the filtration rate and approaches $(1 - \delta d)$.[12]

In order to evaluate the lungs histologically, animals were killed with an overdose of pentobarbital and potassium chloride at the end of the experiment. Gluteraldehyde 2.5% was then instilled into the lungs through an endotracheal tube at a pressure of 25 cm H_2O. After 20 minutes, the hilum of the left lung was clamped. The lung was removed and immersed in glutaraldehyde for 72 hours before sampling. All microscopic sections were stained with hematoxylin and eosin and were interpreted by a pulmonary pathologist (LK) in a blinded fashion. Lung sequestration of neutrophils was quantitated by counting alveolor septal wall neutrophils in 10 randomly chosen high-powered fields (1000 ×). Microscopic fields in proximity to bronchial structures pleura and large vessels were excluded.

Experimental Protocol

Experiments were conducted in anesthetized sheep, placed supine, ventilated with room air at a tidal volume of 15 ml/Kg and rate of 12 to 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 pentobarbital (0.1 mg/Kg/min) and pancuronium (30 μg/Kg/min). Saline, 7 ml/kg/hour was infused throughout 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. Tourniquets were applied as high on both hindlimbs as possible and inflated to 300 mmHg. After 2 hours of ischemia, the tourniquets were removed and the animals monitored for another 2 hours.

Studies were conducted in untreated ischemic animals (n = 10), or in sheep (n = 7) depleted of circulating leukocytes by hydroxyurea (Sigma, St. Louis, MO) (n = 4) 220 mg/Kg I.V. daily over 4–6 days before the study, or by nitrogen mustard (Sigma) (n = 3), 0.4 mg/Kg I.V. given on the sixth and the third day before the study.

In three untreated sheep, after a baseline period of 1 hour, the left atrial balloon was inflated to increase LAP to 18–20 mmHg. This was designed to maximize and stabilize pulmonary vascular surface area in order to interpret changes in $\dot{Q}L$ and protein clearance. The left atrial balloon was kept inflated throughout the experiment. After 1 hour of balloon inflation, the legs were made ischemic. By the time of reperfusion, after 3 hours of increased LAP, a steady state of high $\dot{Q}L$ and filtration independent L/P protein ratio had been achieved.

Sham sheep (n = 3) were subjected to 6 hour of anesthesia in a supine position after which they were killed and the lungs removed for histologic examination.

Results are expressed in the text and figures as mean ± standard error. Differences between means were tested by paired and nonpaired *t*-test. When multiple comparisons were done, the Bonferroni procedure was applied.[13] Significance was accepted if $p < 0.05$.

Animals used in this study were maintained in accordance with the guidelines of the Committee of 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).

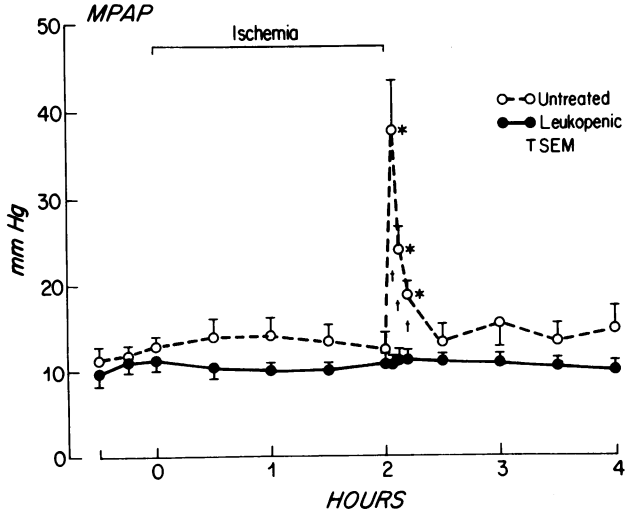


FIG. 1. In untreated ischemic controls 1 minute after tourniquet release, there was a transient rise in mean pulmonary artery pressure (MPAP). This reperfusion-induced pulmonary hypertension was prevented in leukopenic animals. Asterisks indicate significant difference relative to baseline, and crosses indicate significant difference between groups.

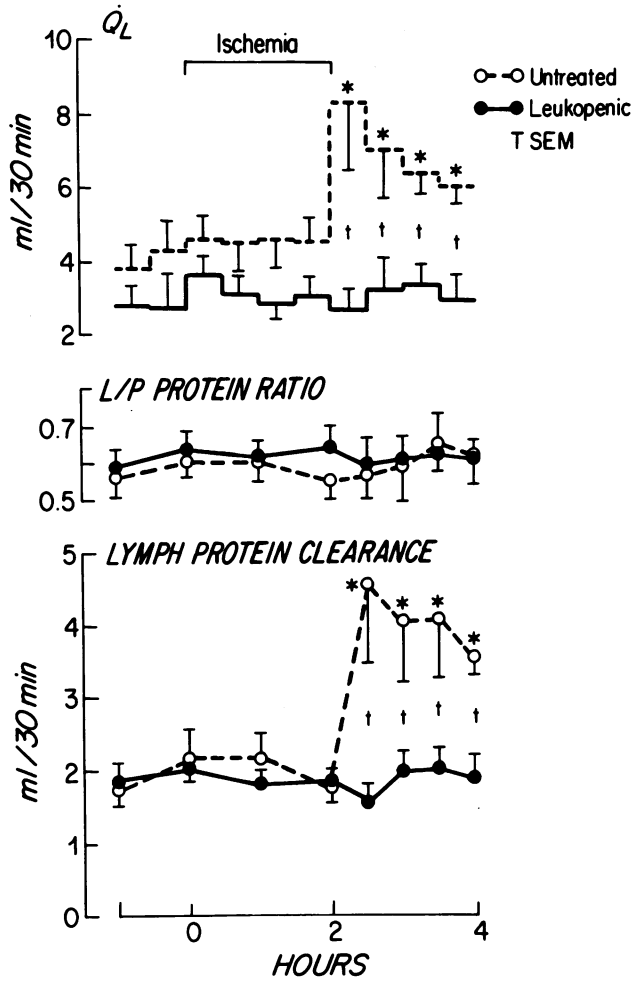


FIG. 2. During reperfusion lung lymph flow ($\dot{Q}L$) doubled and remained elevated for 2 hours, the lymph/plasma (L/P) protein ratio was unchanged, and the lymph protein clearance increased. These changes are consistent with an increased permeability to protein and were prevented by rendering sheep leukopenic. Asterisks indicate significance relative to baseline, and crosses indicate significance between untreated and leukopenic animals.

Results

During the 2 hours of tourniquet ischemia, there were no alterations in cardiopulmonary function, blood counts, or prostanoid levels relative to baseline values. In untreated animals, 1 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 also a transient rise in Pmv from 7 ± 1 to 18 ± 2 mmHg ($p < 0.05$). This rise was temporally related to the increase in MPAP since PAWP was unchanged from baseline value of 4 ± 1 mmHg. Also unaffected were blood gases. Sys-

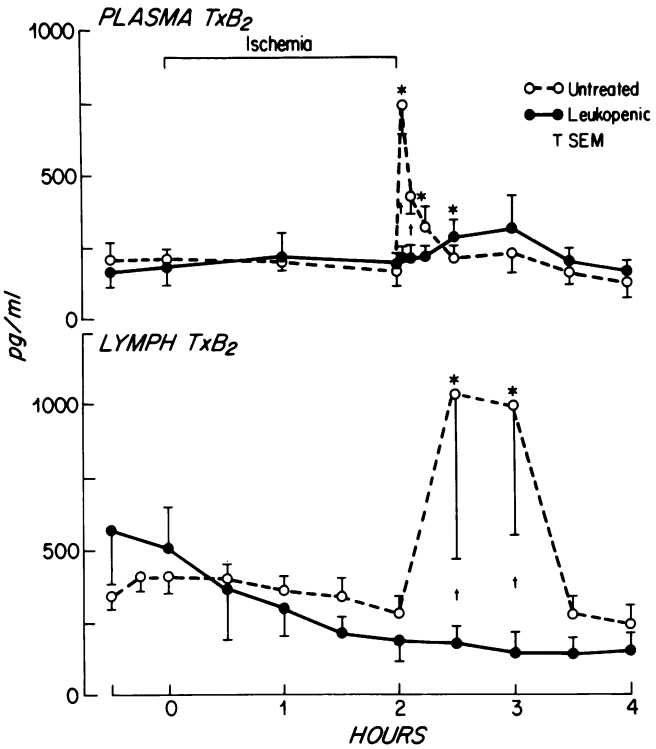


FIG. 3. After 2 hours of bilateral hind limb ischemia, tourniquet release led to an immediate rise in plasma TxB_2 levels. Lung lymph TxB_2 levels also increased and remained elevated for a longer period than in plasma. In leukopenic sheep plasma TxB_2 levels rose slightly 30 minutes after reperfusion. There was no increase in TxB_2 concentration in lung lymph. Asterisks indicate significance relative to baseline, and crosses indicate significance between untreated and leukopenic animals.

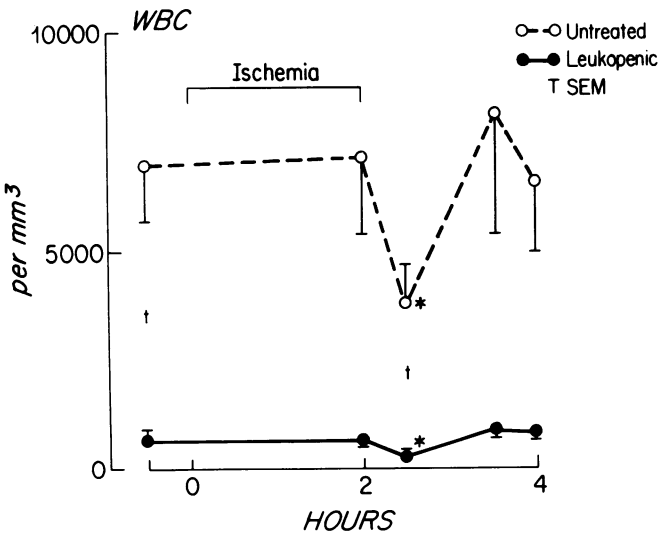


FIG. 4. Upon reperfusion, leukocyte (WBC) counts fell and thereafter returned to preischemic values. Pretreatment with hydroxyurea or nitrogen mustard led to baseline leukopenia. In leukopenic animals, WBC declined further during reperfusion. Asterisks indicate significance relative to baseline, and crosses indicate significance between untreated and leukopenic animals.

temic arterial pressure and cardiac output tended to decline within 5 minutes of reperfusion, but this was not statistically significant.

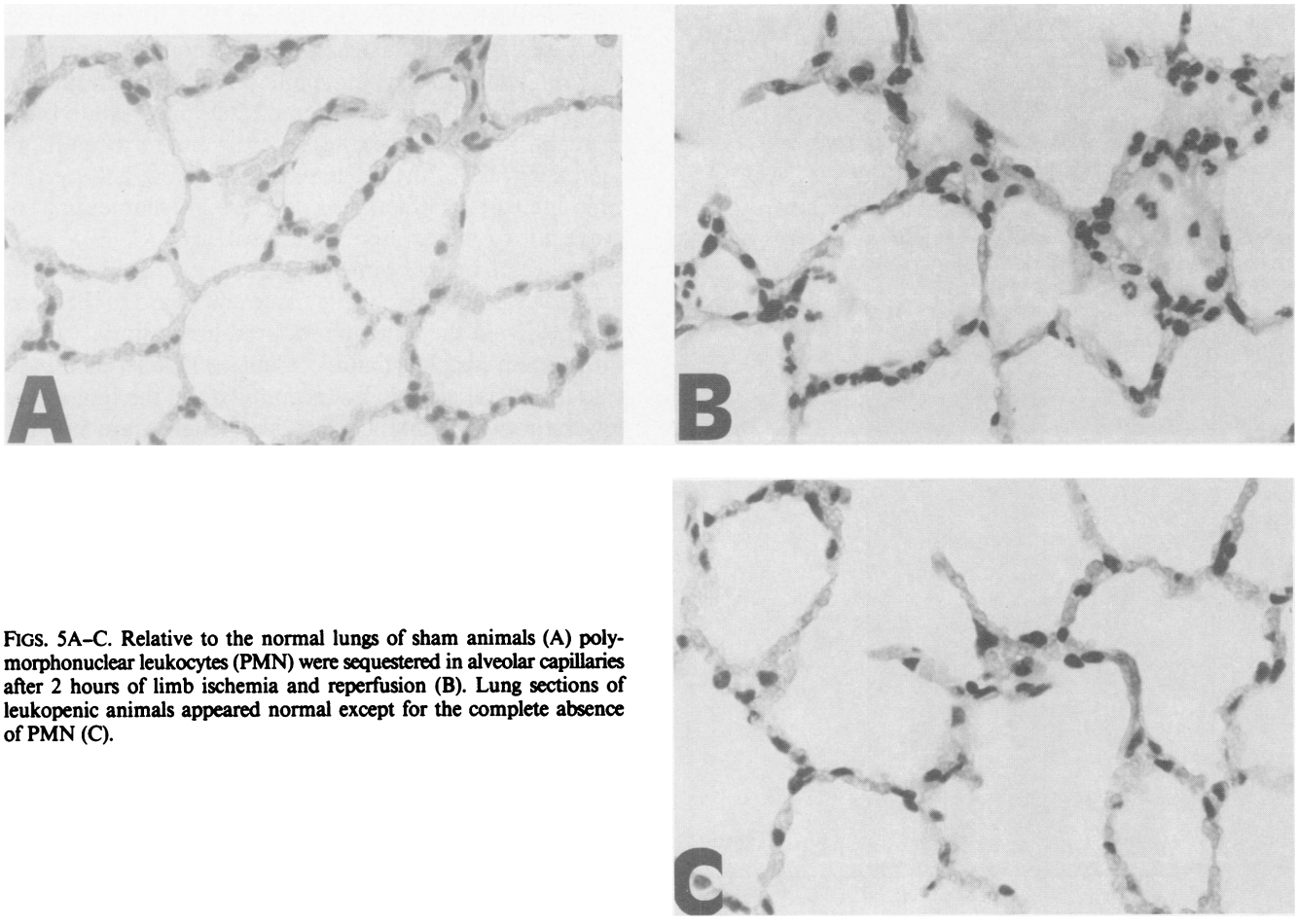
Lung $\dot{Q}L$ increased from a baseline value of 4.3 ± 0.6 to 8.3 ± 1.8 ml/30 minute ($p < 0.05$) 30 minutes after tourniquet release and remained elevated during the 2 hours of reperfusion (Fig. 2). The L/P protein ratio was unchanged from 0.6 ± 0.03, and the lymph protein clearance increased from 2.6 ± 0.4 to 4.6 ± 0.8 ml/30 minutes ($p < 0.05$) (Fig. 2). Plasma TxB_2 levels rose from 211 ± 21 to 735 ± 112 pg/ml ($p < 0.05$) at 2 minutes of reperfusion and returned to baseline levels at 30 minutes (Fig. 3). Lung lymph TxB_2 levels rose from 406 ± 41 to 1005 ± 530 pg/ml ($p < 0.05$) and remained elevated for a more prolonged period than plasma levels (Fig. 3). Plasma and lymph 6-keto-$PGF_{1\alpha}$ levels 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 animal. The average decline during the first hour of reperfusion was from a baseline value of 6853 ± 1149 to $3796 \pm 874/mm^3$ ($p < 0.01$) (Fig. 4). After the second hour, WBC had returned to $6583 \pm 1749/mm^3$. Platelet counts were unchanged by ischemia and reperfusion from their baseline value of $305 \pm 106 \times 10^3/mm^3$. Histologic examination of the lungs revealed accummulations of polymorphonuclear leukocytes (PMN) within alveolar capillaries throughout the lung parenchyma (Figs. 5A–C). Foci of proteinaceous exudate were present within alveolar spaces. Neither platelet nor thrombin microaggregates were seen.

Quantitative PMN counts showed 62 ± 3 PMN/10 high-powered fields (HPF) in dependent and 37 ± 8 PMN/10 HPF in nondependent lung regions, both values significantly higher than 22 ± 3 PMN/10 HPF ($p < 0.05$) in dependent and 16 ± 2 PMN/10 HPF ($p < 0.05$) in nondependent regions of sham animals.

Left atrial balloon inflation in three ischemic sheep led to an increase in MPAP from 12 ± 1 to 20 ± 2 mmHg. Two minutes after tourniquet release, there was a further and transient increase in MPAP to 35 ± 3 mmHg. With balloon inflation, there was a progressive rise in $\dot{Q}L$ and fall in the L/P protein ratio (Table 1; Fig. 6). Thirty minutes before tourniquet release, $\dot{Q}L$ had stabilized at a high flow of 9.8 ± 2.3 ml/15 minutes, while the L/P protein ratio had decreased from 0.70 ± 0.01 to 0.30 ± 0.01. The calculated δd was 0.72 ± 0.02. With reperfusion, $\dot{Q}L$ increased further, and 90 minutes after tourniquet release, it was 11.2 ± 3.6 ml/15 minutes. At the same time, the L/P protein ratio increased to 0.56 ± 0.02, while δd decreased to 0.44 ± 0.02 (Table 1). These data indicate an increase in pulmonary microvascular permeability.

The administration of hydroxyurea or nitrogen mustard resulted in a total leukocyte count of $760 \pm 111/mm^3$ and



FIGS. 5A–C. Relative to the normal lungs of sham animals (A) polymorphonuclear leukocytes (PMN) were sequestered in alveolar capillaries after 2 hours of limb ischemia and reperfusion (B). Lung sections of leukopenic animals appeared normal except for the complete absence of PMN (C).

neutrophil count of 150 ± 80/mm^3. The platelet count was 351 ± 49 × 10^3/mm^3, unchanged from normal animals. Baseline cardiopulmonary function and prostanoid levels were not significantly different in leukopenic sheep relative to ischemic control animals. Leukopenia prevented the reperfusion-induced increases in MPAP ($p < 0.05$), Pmv ($p < 0.05$), $\dot{Q}L$ ($p < 0.05$), and lymph protein clearance ($p < 0.05$) (Figs. 1 and 2). Since the L/P protein ratio was also unchanged from 0.64 ± 0.05, these data indicate that the vascular barrier in leukopenic animals was unaltered by ischemia and reperfusion. Leukopenia also prevented a rise in plasma TxB_2 levels during the first 15 minutes of reperfusion from baseline levels of 119 ± 59 pg/ml, but at 30 minutes levels increased to 288 ± 49 pg/ml ($p < 0.05$) (Fig. 3). The rise in lung lymph TxB_2 levels was prevented ($p < 0.05$) (Fig. 3). Further, plasma and lymph 6-keto-$PGF_{1\alpha}$ concentrations were unchanged from baseline levels of 15 ± 10 and 132 ± 65, respectively. The already low circulating WBC counts decreased even further within the first hour of reperfusion to 360 ± 48/mm^3 ($p < 0.05$) (Fig. 4), while platelet counts were unchanged from baseline values. Lung histology was normal save for the absence of neutrophils in alveolar capillaries (Fig. 5). There was no difference in the pathophysiologic response to ischemia and reperfusion between animals rendered leukopenic by hydroxyurea or nitrogen mustard.

TABLE 1. *Effects of Left Atrial Balloon Inflation on Lung Lymph Dynamics Before and After Ischemia*

		Time of Balloon Inflation	
	Before	3 hours (before reperfusion)	4½ hours (1½ hours after ischemia)
LAP (mmHg)	5 ± 1	20 ± 1	20 ± 2
MPAP (mmHg)	12 ± 1	20 ± 2	21 ± 2
$\dot{Q}L$ (ml/15 min)	2.3 ± 0.5	9.8 ± 2.3	11.2 ± 3.6
L/P	0.70 ± 0.0	0.30 ± 0.01	0.56 ± 0.02
δd	—	0.70 ± 0.02	0.44 ± 0.02

Values are mean ± standard error, LAP is left atrial pressure, MPAP is mean pulmonary artery pressure, $\dot{Q}L$ is lung lymph flow, L/P is lymph plasma protein ratio, δd is the osmotic reflection coefficient.

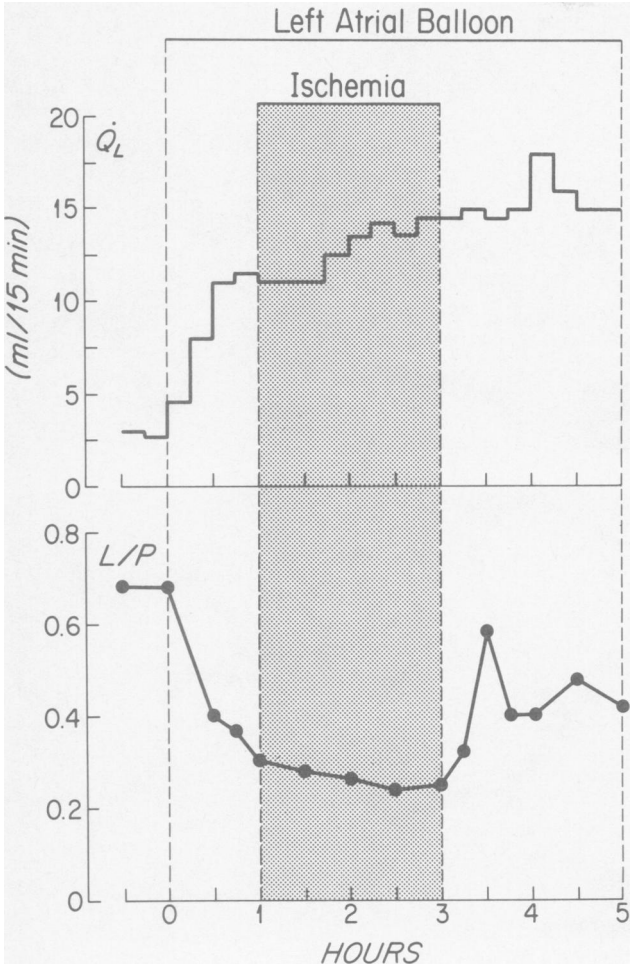


FIG. 6. In a typical experiment, left atrial balloon inflation led to an increase in $\dot{Q}L$ and decrease in the L/P protein ratio. After 3 hours, a new steady state of high $\dot{Q}L$ and a pressure-independent L/P ratio was achieved. Reperfusion then led to a further increase in $\dot{Q}L$. The simultaneous increase in the L/P protein ratio documents increased microvascular permeability to protein.

Discussion

Reperfusion after a 2-hour period of bilateral lower torso ischemia led to lung injury manifest by pulmonary hypertension and increased microvascular permeability. These changes were associated with TxA_2 generation, a decline in circulating leukocytes, and their entrapment in the lungs.

The increase in lung vascular permeability is suggested by a twofold increase in $\dot{Q}L$ with an unchanged L/P protein ratio. Thus, lymph protein clearance increased in proportion to the increase in $\dot{Q}L$. However, interpretation of such lymph data is confounded by the possibility that the rise in $\dot{Q}L$ is due to an increase in lung vascular surface area, a phenomenon related to recruitment of vessels secondary to increased hydrostatic pressure.[14] This interpretation was excluded by using control sheep in which stable increases in surface area and MPAP were achieved by inflation of a left atrial balloon. During the 3 hours of balloon inflation before reperfusion, the L/P protein ratio decreased while $\dot{Q}L$ increased. Both eventually plateaued with $\dot{Q}L$ four times baseline and an L/P protein ratio of 0.3. This L/P ratio is considered filtration pressure independent.[12,14] In this setting, reperfusion led to a further increase in $\dot{Q}L$. Further, the accompanying L/P protein ratio increased while the δd decreased, documenting increased lung microvascular permeability.[12,14]

Earlier studies were interpreted as showing that platelet aggregates from the ischemic tissue embolized to the lungs and mediated the ischemia induced lung injury.[15] However, recent histologic and ^{111}I-labeled platelet data indicate that these cells do not accumulate in the lungs after lower torso ischemia.[1,16] Further, platelets are not the source of TxA_2 in the ischemic setting.[17] Finally, hydroxyurea and nitrogen mustard protect the lungs despite the fact that these agents do not deplete platelets. These considerations indicate that platelets do not have a role in mediating ischemia-induced lung injury.

On the other hand, the protective effect of leukopenia indicates that these cells play a central role in inducing lung injury after remote ischemia. The possibility that hydroxyurea provides protection by a direct action on the vascular barrier[18] rather than its leukopenic effect is unlikely. The short plasma half-life of hydroxyurea, less than 1 hour, excludes the possibility that the drug was circulating during ischemia since the last dose had been given 24 hours before the experiment. Further, in other WBC-dependent lung injuries, neither hydroxyurea[19] nor nitrogen mustard[20] provided protection if neutrophils were not sufficiently depleted. Finally, nitrogen mustard, a drug structurally dissimilar from hydroxyurea, provides the same protection to the vascular barrier, again casting doubt on a local action of these drugs. Nevertheless, the effects of these chemotherapeutic agents on resident lung cells such as macrophages and mast cells are unknown and may be of some protective importance in addition to their action on circulating WBC.

The current data indicate that WBC are directly or indirectly the source of TxA_2 in the ischemic setting. WBC are a potential source of TxA_2.[21] However, thromboxanes may not be generated by WBC themselves but may be a consequence of WBC interaction with the lungs, stimulating extravascular cells such as pulmonary macrophages and mast cells to synthesize TxA_2. The slight although significant increase in plasma TxB_2 during reperfusion in leukopenic sheep is probably due to an incomplete depletion of WBC. Activation of the residual WBC is indicated by their decline after tourniquet release. However, the limited number of cells that could lead to release of vasotoxic agents was insufficient to lead to lung injury.

Neutrophils have several potent mechanisms to induce tissue injury. They can release lysosomal enzymes and oxygen-derived free radicals (OFR).[2] Involvement of the latter agents is suggested by the protective effect of OFR

scavengers such as superoxide dismutase and catalase (unpublished observation). Neutrophils can also produce and release arachidonic acid derivatives, especially leukotriene (LT) B_4 and TxA_2.[2,21] The WBC–TxA_2 interaction is of special importance. TxA_2 has been shown to be an essential mediator of reperfusion injury.[16,22] Thus, pretreatment with a Tx synthetase inhibitor prevented reperfusion pulmonary hypertension increase in permeability as well as lung leukosequestration.

Thromboxane A_2 can act directly on the endothelial cell (EC) cytoskeleton, leading to actin microfilament disassembly, widening of interendothelial tight junctions, and increased permeability.[23] In addition, TxA_2 can act indirectly by promoting neutrophil adhension[24] and enhancing PMN diapedesis through EC monolayers.[25] These events can result in decreased barrier function.

We hypothesize that WBC contained in the limbs during ischemia are involved in the cascade of events that eventuates in the synthesis of a chemoattractant that is released into the systemic circulation during reperfusion, leading to lung leukosequestration and injury. Secondary to their rheologic properties, WBC contained in the ischemic tissue exhibit enhanced adherence, which may by itself activate them to release chemoattractants such as LTB_4.[26,27] In addition, superoxides and other OFR can be generated as a consequence of ischemia in myocytes, endothelial cells, and macrophages as well as WBC.[28] These OFR can in turn activate the arachidonic acid cascade[29] in adjacent cells leading to the synthesis of agents such as LTB_4 and TxA_2 that are themselves vasotoxic and can also recruit more WBC to the ischemic site. This may amplify the local injury. Upon reperfusion, sufficient chemoattractants such as LTB_4 may be released into the circulation, causing lung leukosequestration and injury.[3,4] LTB_4 and TxA_2 are most likely the chemoattractants mediating this injury since their blockade with a lipoxygenase or Tx synthetase inhibitor prevents not only the lung injury after ischemia but also the entrapment of neutrophils in pulmonary capillaries.[1,3,16,22]

In summary, these findings indicate that the lung injury after a remote ischemic event is mediated by leukocytes. Secondly, these cellular elements are involved in TxA_2 synthesis, an agent also intimately involved with lung injury.

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