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BRONCHOVASCULAR CUFF FORMATION AND LUNG LYMPH FLOW IN EDEMA FORMATION OF ANESTHETIZED SHEEP

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ABSTRACT

The relationship between bronchovascular cuff formation and lung lymph flow in hydrostatic edema was evaluated. After a balloon was inserted into the left atrium to increase left atrial pressure for 5 hrs, peripheral lung tissues were resected for analysis of the wet-dry ratio and cuff formation. The degree of cuff formation was expressed as the cuff ratio (outer diameter of cuff / outer diameter of microvessel or airway) in three size categories: 80-200, 200-400, and 400-750 µm in diameter. The amount of excess lung lymph (Ex LL) for 5 hrs was calculated from the recorded data for the whole lymph flow wave. The wet-dry ratio showed a significant correlation with ΔLAP and lung lymph flow increased significantly (flow rate, 0.67 ± 0.46 ml/min (mean ± SD); Ex LL, 56.4 ± 47.6 ml). Cuff formation was found at all levels of the bronchovascular tree, with a larger cuff ratio (>1.3) observed at arteries and veins of 80-200 &m in diameter, but a significant correlation with Ex LL was found only for arteries of 80-200 µm. Fluid accumulation in lung interstitium first occurred at smaller extra-alveolar arteries even under mild hydrostatic pressure elevation with a significant increase in lymph flow.

Keywords: lung lymph, bronchovascular cuff, interstitial pulmonary edema, hydrostatic pressure edema, lung fluid balance, sheep

Interstitial pulmonary edema leads to alveolar flooding, which is a condition that is difficult to manage clinically. Pulmonary edema needs to be identified at an early stage to prevent disease progression. During development of pulmonary edema, extravascular lung fluid is present in two anatomical spaces: in the interstitial space of a developing bronchovascular cuff and in lymphatics draining into the systemic circulation. The relationship between fluid volume in the lung and lung lymph flow has been examined in several experimental models. Pine et al (1) demonstrated a significant increase in extravascular lung water accompanied by large increases in lung lymph flow but with only mild interstitial swelling by producing increased permeability edema with alpha-naphthylthiourea in canine experiments. Brigham et al (2) showed that only small changes in extravascular lung water volume were accompanied by large increases in lung lymph flow in sheep given pseudomonas aeruginosa, and also found a normal postmortem wet-dry ratio after extremely high lymph flow for 1-9 hrs. In the current study we reanalyzed previously published data from a study demonstrating a new method for evaluation of lung lymph flow rate in anaesthetized sheep (3) by examining the relationship between interstitial fluid accumulation and lung lymph flow in increased hydrostatic pressure edema without alveolar flooding.

Analysis of swelling of interstitial tissue around pulmonary vessels or bronchi is a useful method for estimation of fluid accumulation in the interstitial space. Staub et al (4) determined the sequence of fluid accumulation during pulmonary edema formation using a rapid-freeze technique in canine lungs and showed that fluid accumulation first occurred in the perivascular and peribronchial interstitium, and accumulated fluid then flooded into the alveoli. The fluid-filled interstitium around the bronchovascular space is called a fluid cuff and is used in morphometric analysis of pulmonary edema. Quantitative analysis of the bronchovascular cuff in pulmonary edema by Michel et al (5-7) showed a preferential distribution of interstitial fluid accumulation in relatively severe pulmonary edema. We performed an additional morphometric study with a similar anatomical classification to that used by Michel et al.

Measurement of lung lymph flow has been performed widely by cannulation or collection of the draining lymph from the lungs in large animals (1,2). We have recently developed a new method to measure lung lymph flow in sheep (3), using an approach that preserves the normal structure of all the pathways functioning in lung lymph drainage and gives a 3- to 6-fold greater baseline lymph flow rate compared to values obtained with the cannulation method. We believe that this method gives an accurate volume flow, and here we reanalyzed data from animals reported in a previous study (3), using a new calculation to investigate the correlation between increased lymph flow volume and other indices of pulmonary edema formation, including the degree of bronchovascular cuff formation.

MATERIALS AND METHODS

Animals and General Methods

General anesthesia was initiated with intramuscular injection of ketamine hydrochloride (10 mg/kg) in six crossbreed sheep of either sex (body weight, 40-50 kg). Endotracheal intubation was performed after intravenous injection of thiopental sodium (5 mg/kg). Anesthesia was maintained with 0.5-1.0% halothane and 50-55% oxygen in N_2O, using a positive-pressure ventilator (Harvard Pump Model No. 607) with a tidal volume of 450-500 ml, and at a respiratory rate of 15-20 /min under muscle relaxation by pancuronium bromide (0.05 mg/kg/h). The concentration of halothane was adjusted to maintain the mean arterial blood pressure at about 100 mmHg during the experiments. A catheter was inserted into the right common carotid artery and connected to a pressure transducer (MPU-0.5A, Nihon Kohden, Tokyo) for measurement of arterial blood pressure (ABP) continuously and for arterial blood gas analysis every hour. A Swan-Ganz catheter was inserted into the right external jugular vein and connected to a low-pressure transducer (LPU-0.1A, Nihon Kohden) for measurement of central venous pressure (CVP), pulmonary arterial pressure (PAP), and pulmonary capillary wedge pressure, which is approximately equal to the left atrial pressure (LAP). The zero level of all pressures was adjusted to the pressure at the level of the cranial vena cava at its entrance to the heart. Pressure data were recorded using a multi-channel polygraph system (RM-6000, Nihon Kohden) and were also recorded on a personal computer. Drip infusion of Ringer's lactate was maintained at 200 ml/hr during surgery and 100 ml/hr during measurements. The experimental protocols were approved by the Animal Experimentation Committee of the University of Tsukuba.

Lung Lymph Flow Measurement

Lung lymph flow was measured as we have described previously (3). Briefly, right 5th and 8th intercostal thoracotomy was performed, and a small amount of blue dye (diluted Evans blue) was injected into the caudal mediastinal lymph node (CMN)

through the 8th intercostal space. Flow of the blue dye was observed in the efferent ducts of the CMN and the thoracic duct through the 5th intercostal space. After confirming the position of the last junction of the efferent ducts of the CMN with the thoracic duct, an ultrasound transit-time flow probe (Model H2SB, Transonic Systems Inc., Ithaca, NY) was attached to the thoracic duct just downstream of the junction. Thoracic duct flow was obstructed at just above the diaphragm. After the obstruction, the thoracic duct flow measured at the probe site is the total outflow from the CMN containing the lymph sourced from the lung and the peritoneal cavity. To exclude abdominal lymph, we cauterized the bilateral diaphragm. The flow probe was connected to an ultrasound transit-time flow meter (Model H207, Transonic Systems Inc.), and the lymph flow signal was recorded digitally every 1 second on a computer disk.

Experimental Protocol

To establish hydrostatic pulmonary edema, experiments were performed using the classic method. A balloon catheter (Foley Catheter 8 Ch/Fr, Bard, West Sussex, UK) was introduced into the left atrium (LA) through a branch of the pulmonary vein. The baseline thoracic duct flow was measured for 10 min before the thoracic duct was obstructed just above the diaphragm. Baseline measurement of lung lymph flow was then performed for 20 min. After these measurements, the balloon in the LA was inflated until LAP increased to about 10 cmH_2O above baseline. Inflation was maintained for 5 hours and then the animals were sacrificed. During the experimental period, the oxygen concentration of the inspired gas was increased appropriately to maintain arterial blood gas within the normal range.

Peripheral lung tissue samples (1.5 x 1.5 x 1 cm) of the right middle lobe were excised at the end of the experiment. The samples were passively drained of blood, immediately weighed, and then placed in a desiccating

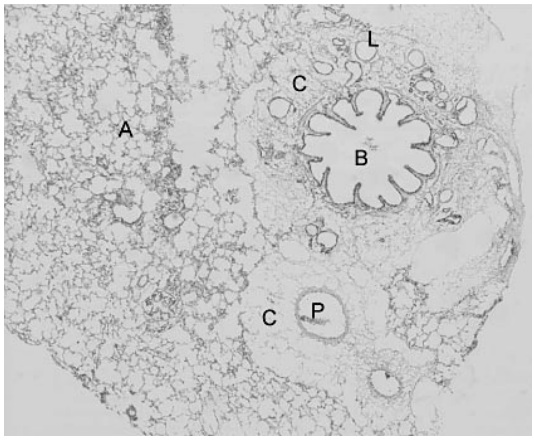

Fig. 1. An example of a histological specimen showing fluid-filled cuffs. Alveoli (A), bronchus (B), fluid cuffs (C), pulmonary artery (P), lymphatic (L). Hematoxylin and eosin staining x100.

oven at room temperature until constant dry weight was reached (8). The ratio of the wet to dry weight was used to quantify the lung water content. For histological analysis, a peripheral lung tissue sample (4 x 4 x 3 cm) of the right lower lobe was excised at the end of the experiment and immediately frozen-fixed in liquid nitrogen for cuff analysis (5). The frozen specimens were sliced in a cryostat (15 slices from each tissue sample) and stained with hematoxylin and eosin. All slices were examined for histological evidence of pulmonary edema, such as alveolar flooding and cuff formation around microvessels or airways (*Fig. 1*).

Calculation of "Excess Lung Lymph"

Recorded lymph flow waves were analyzed by a computer program (Flowtrace, Transonic Systems Inc.) to obtain the lymph flow volume (ml) by integration over an appropriate period. We postulate that "excess lung lymph" (Ex LL) is the amount of drained lymph volume over baseline through lymphatics, out of the net filtered fluid induced by pressure stress (*Fig. 2*). Ex LL was calculated in each sheep as:
Ex LL = Net LL — Baseline LL

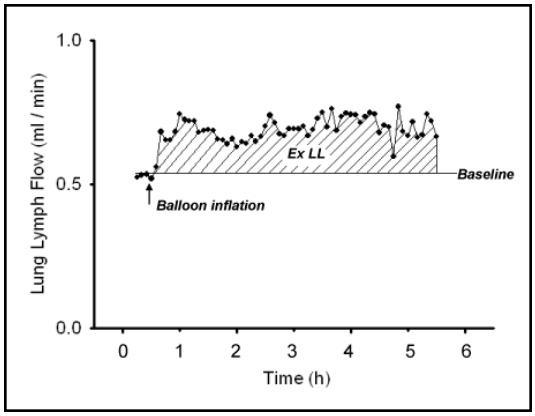

Fig. 2. Excess lung lymph (Ex LL) in each sheep was calculated by integration of the recorded lymph flow wave to give the area above baseline (area shown by oblique lines) in this study. The graph shows the time course of lung lymph flow (mean of 6 sheep, SD bars omitted), as reported in a previous study (23). After baseline measurement of lung lymph flow for 20 minutes, a balloon was inflated in the left atrium and kept inflated until the end of the experiment. Lung lymph flow increased significantly 10 minutes after balloon inflation and this increase was maintained until the end of the experiment ($p < 0.05$, compared with baseline).

Net lung lymph (Net LL) was obtained by integration of the whole lymph wave recorded for 5 hrs after inflation of the balloon in the LA. Baseline lung lymph (Baseline LL) is the corresponding baseline value for the same period (5 hrs) in the same sheep.

Quantitative Analysis of Cuff Formation

All microvessels and airways of more than 80 μm in diameter were analyzed on all slides prepared for microscopic examination. The cuff ratio was calculated by dividing the diameter of the outer border of a microvessel or airway, including fluid-containing loose connective tissue (adventitia), by the diameter contoured by the external lamina of the microvessel (external border of the media) or by the subepithelial basement membrane of the airway:
Cuff ratio = Diameter of cuff / Diameter of microvessel or airway

If the cut of the microvessel or airway gave an elliptical shape, the longest diameter was measured. Both the diameter of the cuff and that of the microvessel or airway were measured on the same axis. In some cases, there was fluid collection in the adventitia between the airway and the associated pulmonary artery. This is referred to as a bronchovascular bundle (6), and such bundles were not included in the measurement of the diameter of the outer border of the cuff.
The degree of bronchovascular cuff formation was analyzed in three groups based on the level in the bronchovascular tree: the alveolar sac, which has an estimated diameter of < 200 μm in human lung, was assumed to be the smallest airway likely to develop a bronchial cuff; airways of diameter 200-400 μm, referred to as alveolar ducts or respiratory bronchioles; and airways of diameter > 400 μm, referred to as respiratory or terminal bronchioles (9-11). Thus, airways were separated into three sizes based on anatomical classification. Similarly, pulmonary arteries and veins were separated into the same three size categories. Pulmonary arteries usually branch along with airways, and in dogs Michel (12) has shown that all arteries > 100 μm in diameter are accompanied by airways but 20% of arteries of 51-100 μm in diameter are surrounded by alveoli without airways. To distinguish arteries from veins, microvessels of > 80 μm in diameter were considered to be pulmonary arteries if they were associated with airways, and pulmonary veins if they existed in solitary with surrounding alveoli or connective tissue.

Statistics

The significance of differences in hemodynamic parameters and lymph flow from baseline was analyzed by one-way ANOVA with Fisher's protected least significant difference test. Other comparisons were performed by paired or unpaired *t-test* as appropriate with significance at $p < 0.05$ (StatView 4.5, SAS Institute Inc., Cary, NC).

TABLE 1
Summary of Data for Indices of Pulmonary Edema

	Sheep 1	Sheep 2	Sheep 3	Sheep 4	Sheep 5	Sheep 6
ΔLAP (cmH_2O)	8.7	13.2	9.8	7.2	10.7	14.5
Ex LL (ml)	57.1	57.2	70.2	11.2	5.7	137.3
Wet-dry ratio	5.74	6.98	5.13	5.78	6.90	7.51

ΔLAP: Increased left atrial pressure (LAP), calculated from mean LAP during balloon inflation in the left atrium - mean LAP at baseline. Ex LL: Excess lung lymph, calculated from net lung lymph flow during balloon inflation in the left atrium for 5 hrs - corresponding baseline lung lymph flow (*Fig. 1*). Wet-dry ratio: Peripheral tissues were excised after balloon inflation in the left atrium for 5 hrs.

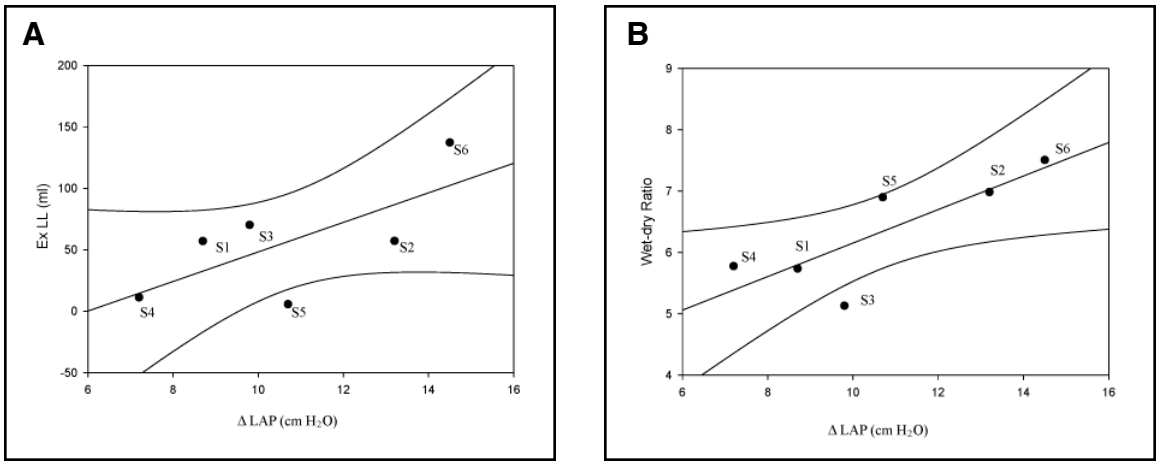

Fig. 3. Increased left atrial pressure (ΔLAP) was calculated from the mean LAP for 5 hrs after balloon inflation in the left atrium - the mean LAP for 20 min at baseline. Each dot in the figure represents an individual sheep (S1-S6). (A) Excess lung lymph (Ex LL) was calculated by integration of the recorded lymph flow wave during inflation of a balloon in the left atrium for 5 hrs - the baseline amount of lymph over the same period. There was no correlation between Ex LL and ΔLAP, as shown by the regression line (r = 0.69) and 95% confidence interval. (B) Wet-dry ratio was measured from excised lung, and this parameter showed a significant positive correlation (p < 0.05) with ΔLAP, as shown by the regression line (r = 0.82) and 95% confidence interval.

The significance of a correlation between two parameters was evaluated by Pearson's correlation coefficient test at $p < 0.05$ (Statcel 2, OMS Publisher, Saitama, Japan). Hemodynamic and lymph flow data are shown as means ± standard deviation (SD) during the baseline and balloon-inflated periods.

RESULTS

Surgical and instrumental preparation was performed under stable conditions for each animal. ABP did not change during the experimental period (systolic pressure 120 ± 4 mmHg, diastolic pressure 74 ± 6 mmHg).

TABLE 2
Summary of Data for Cuff Ratio of Microvessels and Airways

	Size category (diameter, μm)								
	80 - 200			200 - 400			400 - 700		
	Cuff ratio	(n)	(mean diam.)	Cuff ratio	(n)	(mean diam.)	Cuff ratio	(n)	(mean diam.)
1) Pulmonary artery									
Sheep 1	1.28±0.12	(15)	(147±31)	1.17±0.10	(10)	(287±49)	1.16±0.08	(9)	(463±55)
Sheep 2	1.39±0.23	(15)	(147±34)	1.13±0.09	(6)	(298±45)	1.24±0.06	(7)	(479±80)
Sheep 3	1.37±0.23	(9)	(149±34)	1.17±0.03	(11)	(254±50)	1.17±0.06	(7)	(485±60)
Sheep 4	1.36±0.19	(11)	(146±27)	1.18±0.06	(8)	(256±42)	1.23±0.12	(8)	(492±54)
Sheep 5	1.25±0.08	(11)	(143±25)	1.20±0.09	(11)	(280±58)	1.16±0.08	(7)	(503±81)
Sheep 6	1.54±0.50	(6)	(154±24)	1.26±0.17	(15)	(304±50)	1.27±0.19	(6)	(466±57)
Mean (n=6)	1.37±0.10			1.18±0.05			1.21±0.04		
2) Pulmonary vein									
Sheep 1	1.43±0.28	(7)	(128±40)	1.12±0.05	(12)	(283±62)	1.08±0.02	(6)	(503±90)
Sheep 2	1.33±0.18	(10)	(153±31)	1.23±0.12	(10)	(272±66)	1.19±0.09	(7)	(519±70)
Sheep 3	1.40±0.17	(12)	(145±32)	1.20±0.09	(7)	(301±73)	1.19±0.07	(10)	(456±39)
Sheep 4	1.39±0.17	(11)	(143±27)	1.22±0.07	(7)	(285±60)	1.17±0.08	(9)	(492±81)
Sheep 5	1.33±0.13	(10)	(160±32)	1.21±0.11	(7)	(295±59)	1.21±0.16	(6)	(444±31)
Sheep 6	1.43±0.36	(11)	(152±27)	1.29±0.17	(10)	(299±57)	1.17±0.05	(7)	(509±54)
Mean (n=6)	1.39±0.05			1.21±0.05			1.17±0.05		
	100 - 200			200 - 400			400 - 750		
3) Airway									
Sheep 1	1.17±0.08	(13)	(160±21)	1.13±0.06	(21)	(279±55)	1.16±0.03	(7)	(601±67)
Sheep 2	1.22±0.11	(9)	(149±25)	1.14±0.08	(23)	(276±48)	1.15±0.06	(7)	(572±71)
Sheep 3	1.20±0.09	(9)	(154±30)	1.12±0.05	(26)	(294±56)	1.15±0.05	(7)	(501±30)
Sheep 4	1.18±0.09	(15)	(152±24)	1.14±0.05	(22)	(291±52)	1.15±0.03	(6)	(601±89)
Sheep 5	1.23±0.11	(13)	(144±21)	1.16±0.06	(23)	(293±52)	1.15±0.01	(6)	(594±108)
Sheep 6	1.19±0.09	(12)	(152±24)	1.14±0.07	(18)	(309±60)	1.21±0.22	(10)	(569±97)
Mean (n=6)	1.20±0.02			1.14±0.01			1.16±0.02		

Mean values with standard deviations for calculated cuff ratios in each size category for each sheep are shown, with the mean cuff ratio of the 6 sheep are shown on the bottom row. Numbers in parentheses indicate numbers of analyzed microvessels or airways in each category for each sheep (n) and the mean values with standard deviations are diameters of microvessels or airways in each category for each sheep (mean diam.).

After inflation of the balloon in the LA, there were significant increases in mean PAP (20.5 ± 0.1 vs. 26.6 ± 1.4 cmH_2O, $p < 0.05$) and LAP (4.9 ± 0.4 vs. 16.7 ± 1.4 cmH_2O, $p < 0.05$). An increased left atrial pressure (ΔLAP) was calculated from the difference in mean LAP values during balloon inflation and at baseline (*Table 1*), with a mean ΔLAP (n = 6 sheep) of 10.7 ± 2.7 cmH_2O. CVP did not change during the experiments (1.8 ± 4.6 cm H_2O), and arterial blood gas was maintained within the normal range.

The baseline thoracic duct flow was 2.49 ± 1.11 ml/min. Lung lymph flow at baseline and after balloon inflation in the LA are shown in *Fig. 2* as the time course of mean values at 5-min intervals for 6 sheep (the numbers on the time axis begin from the start of the flowmeter signal). The mean lung lymph flow at baseline was 0.56 ± 0.43 ml/min (at 20 min), and this increased significantly ($p < 0.05$) to 0.67 ± 0.46 ml/min (at 5 hrs) after balloon inflation in the LA. Calculated Ex LL values are shown in *Table 1* with the

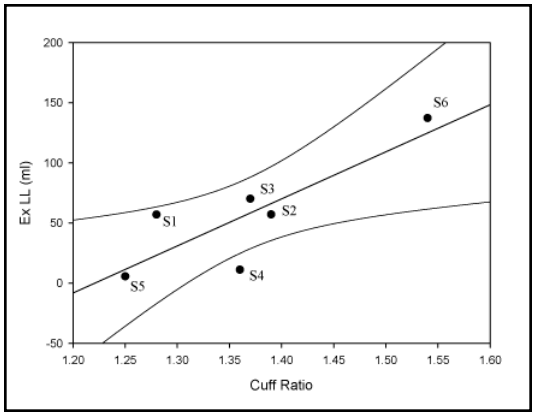

Fig. 4. A significant positive correlation ($p < 0.05$) was found between the cuff ratio of pulmonary arteries of 80-200 µm in diameter and excess lung lymph (Ex LL), as shown by the regression line ($r = 0.84$) and 95% confidence interval. Each dot represents an individual sheep (S1-S6).

mean Ex LL over 6 sheep at 56.4 ± 47.6 ml. There was no significant correlation found between Ex LL and ΔLAP (*Fig. 3A*). The wet-dry ratios are shown in *Table 1* with the mean wet-dry ratio at 6.34 ± 0.92. There was a significant positive correlation between wet-dry ratio and ΔLAP (*Fig. 3B*, $p < 0.05$).

Calculated cuff ratios are shown in *Table 2*. Differences between cuff ratios for different artery, vein or airway size categories could not be calculated because the amount of fluid accumulation was not measured directly. The ratio of the diameter of the formed cuff to the microvessel or bronchus was measured but this cuff ratio does not reflect the amount of fluid accumulation precisely, compared to the quantitative morphometry used by Michel et al (5-7). However, we were able to examine differences in cuff ratio between arteries and veins of the same size because the ratio is expressed as the expanded diameter of the cuff. We found no significant differences between these cuff ratios (*Table 2*). Regarding the correlation between cuff ratio and Ex LL in each sheep, a significant positive correlation was found only for pulmonary arteries with a diameter of 80-200 µm (*Fig. 4*, $p < 0.05$). For other size categories of microvessels or airways, there were no correlations between cuff ratio and Ex LL. Similarly, there was no significant correlation between cuff ratio and wet-dry ratio, or between cuff ratio and ΔLAP.

Histological examination of specimens collected at the end of the experiments revealed no alveolar edema. Dilated lymphatics were found in the cuff around the larger airways.

DISCUSSION

We aimed to achieve a moderate elevation of LAP, although ΔLAP varied over a relatively wide range among the sheep (*Table 1*). The increase in lung lymph flow in our study was comparatively higher than those obtained in previous studies (13- 16). Lo et al (16) obtained a high lung lymph flow (0.66 ml/min) with a greatly increased LAP (ΔLAP; 23.1 cmH_2O), and Conhaim et al (14) achieved a high baseline flow (0.19 ml/min) using a cannulation method and obtained triple the baseline flow with a relatively moderate elevation of LAP (ΔLAP; 14.0 cmH_2O). Both the baseline flow and increased flow in our experiments were higher than in previous studies (13-16), despite a moderate ΔLAP, which suggests that our method measures the amount of draining lung lymph with fewer artifacts (3).

Ex LL is the amount of lymph drainage over the baseline level caused by LAP elevation, and the wet-dry ratio reflects the accumulated fluid volume in the interstitium of the whole lung. We found no correlation between Ex LL and ΔLAP (*Fig. 3A*). This was an unexpected result because this correlation should be present with increased hydrostatic pressure edema. However, there was a significant correlation between wet-dry ratio and ΔLAP (*Fig. 3B*). This result suggests that the wet-dry ratio could be a sensitive index for fluid filtration when hydrostatic pressure elevation (ΔLAP in this study) is moderate. Further elevation of LAP would have produced a significant correlation with Ex

LL. It has also been proposed that Ex LL might be more dependent on the total driving force in the equation for fluid filtration derived by Starling (17). Nakahara et al (18) demonstrated that the percentage increase in lymph flow in mild hydrostatic edema is significantly correlated with a net pressure gradient comprising the hydrostatic and oncotic pressure gradients as in Starling's equation. Therefore, a more precise future study is required for accurate measurement of lymph and plasma protein concentrations together with lymph-hemodynamic data (19).

No liquid was found in alveoli in histology specimens obtained at the end of the study and arterial blood gas was kept within the normal range throughout the experiments, which suggests that alveolar flooding did not occur. Lung lymph flow increased significantly from the baseline level from the beginning of LA balloon inflation until the end of the experimental period (*Fig. 2*). However, the moderate wet-dry ratio indicates interstitial fluid accumulation (*Table 1*), and peribronchovascular cuff formation developed evenly in microvessels and airways in all size categories (*Table 2*). Therefore, we conclude that peribronchovascular cuff formation acts together with lymphatic drainage to maintain lung function for as long as possible during an increase in hydrostatic pressure. In other words, interstitial fluid accumulation might function as a safety factor to prevent lung insufficiency produced by increased fluid filtration.

Pulmonary microcirculation, including lymph circulation in the lung interstitium, must be preserved to maintain homeostasis, and such preservation depends on large interstitial compliance (20,21). Well-expanded lymphatics are observed in a fluid-filled interstitium, especially around larger airways, and these lymphatics are continuations from juxta-alveolar lymphatics (22,23), the most peripheral lymphatics for draining interstitial fluid. Bhattacharya (20) suggested that a pressure gradient from alveolar wall junctions to the microvessel adventitia serves as a driving force for liquid removal from the perimicrovascular space toward lymphatics. This pressure gradient is present in normal interstitium and increases with development of interstitial edema (24). Therefore, fluid removal from lung interstitium may start from the beginning of fluid filtration.

Morphometric studies of pulmonary edema based on cuff formation have been performed by Michel et al (5-7) based on earlier qualitative and quantitative studies of the structure of normal canine pulmonary vasculature by Michel (12). We followed the anatomical and pathological criteria used in these studies (6,12) to identify the type of microvessels and the border of the bronchovascular cuff. Since we did not calculate the ratio of the fluid cuff area, but rather the ratio of the fluid cuff diameter, we were unable to compare cuff ratios between microvessels or airways of different size. We were able to compare the cuff ratio between arteries and veins of the same size but we found no significant differences for any of the three size categories (*Table 2*). However, Michel et al demonstrated that fluid accumulates preferentially around arteries compared with veins (7). This discrepancy with our results might be due to the severity of edema since the mean wet-dry ratios in our experiments was 6.34 ± 0.92, compared to 11.66 ± 0.84 in their model. Alternatively, the difference in results might be due to the thick adventitia of a normal pulmonary vein (12), which increases the outer diameter of the cuff of the vein. Since it is difficult to distinguish the fluid portion from the normal adventitia, the absolute fluid content in the cuff might be less in a vein than in an artery of the same size category. Therefore, fluid accumulation might progress more in the arteries as found in Michel's work (5-7).

Several previous studies support our results showing no difference in cuff ratios between arteries and veins of the same size. It has been suggested that the venous side might have the same filtration characteristics as the arterial side, and this possibility was

confirmed by Gropper et al in isolated dog lung lobes under zone 1 conditions (25). They further determined that the combined arterial and venous extra-alveolar segments contribute approximately 25% of the total filtration. Luchtel et al (26) have also suggested that extra-alveolar veins are leaky, and the venous cuff is contiguous with the periarterial cuff in zone 1 isolated rabbit lungs. A direct micropuncture study of larger pulmonary microvessels (27) indicated that the hydrostatic pressure of the vein downstream from 450 μm in diameter increased in parallel with LAP elevation, suggesting that cuff formation on the venous side might develop when LAP is increased.

The mean cuff ratios of airways were small in all size categories (*Table 2*), but there were several cuffs containing a large amount of fluid around airways of 400-750 μm in diameter (e.g., in sheep 6, *Table 2*). This suggests that filtered fluid moved to the proximal portion through the interstitial space around peripheral microvessels where the fluid was filtering. Previously, fluid accumulation around airways of more than 400 μm in diameter has been observed in moderate to severe pulmonary edema under conditions of increased permeability (6,28) and at increased hydrostatic pressure (7). Our confirmation of a fluid cuff around airways of more than 400 μm in diameter was obtained with mild hydrostatic pulmonary edema *(Table 1*). Therefore, fluid movement along the peribronchovascular interstitium might occur even at low ΔLAP with the high lung lymph flow achieved in our study (*Fig. 2, Table 1*). As a driving pressure for fluid movement, Bhattacharya et al (24) measured the interstitial pressure gradient from the alveolar wall junctions to the hilum under conditions of different severity of edema and demonstrated that the pressure gradient increased with the severity of edema.

Many studies have shown that the interstitial fluid is filtered mostly from extra-alveolar vessels (25,26,29-31), although fluid accumulation in the air-blood barrier without any fluid appearance in the extra-alveolar space has also been reported in mild interstitial edema (32). Our data suggest that interstitial fluid accumulation first occurs in pulmonary arteries of 80-200 μm, with larger microvessels then forming interstitial edema, since the arteries showed a larger cuff ratio and a good correlation with Ex LL (*Fig. 4*), whereas larger microvessels showed a smaller cuff ratio and no correlation with Ex LL. Thus, we were able to determine a more specific role for extra-alveolar vessels, which are classically known as fluid filtration sites during early high-pressure edema (4). Parker et al (33) showed significantly higher hydraulic conductance in pulmonary artery conduit vessels than in pulmonary microvessels in cultured cell monolayers, and our model may also allow determination of anatomical-specific regions of pulmonary artery conduit vessels.

In conclusion, mild elevation of LAP created a different severity of pulmonary edema in individual sheep. Among the indices of pulmonary edema, wet-dry ratio and ΔLAP showed a good correlation. To estimate fluid removal from the lung interstitium by the interstitium itself and lymphatics, quantitative assessment of the bronchovascular cuff and a new measurement of lung lymph flow were performed. The cuff ratio at a pulmonary artery of 80-200 μm in diameter and Ex LL showed a significant positive correlation. The extra-alveolar small pulmonary artery might be the initial area of interstitial pulmonary edema. We suggest that full initial lymphatic drainage and interstitial fluid accumulation function as safety factors to prevent lung insufficiency during development of hydrostatic pulmonary edema.

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