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Structural and Functional Evidence for the Scaffolding Effect of Alveolar Blood Vessels

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Abstract

A contribution of pulmonary blood distension to alveolar opening was first proposed more than 100 years ago. To investigate the contribution of blood distension to lung mechanics, we studied control mice (normal perfusion), mice after exsanguination (absent perfusion) and mice after varying degrees of parenchymal resection (supra-normal perfusion). On inflation, mean tracheal pressures were higher in the bloodless mouse (4.0 ± 2.5 cmH₂O); however, there was minimal difference between conditions on deflation (0.7 ± 0.9 cmH₂O). To separate the peripheral and central mechanical effects of blood volume, multi-frequency lung impedance data was fitted to the constant-phase model. The presence or absence of blood had no effect on central airway resistance ($p > .05$). In contrast, measures of tissue damping (G), tissue elastance (H) and hysteresivity (η) demonstrated a significant increase in bloodless mice relative to control mice ($p < .001$). After varying amount of surgical resection and associated supra-normal perfusion of the remaining lung, there was an increase in G and H. Although the absolute difference in G and H increased with the amount of parenchymal resection, the proportional contribution of blood was identical in all conditions. The presence of blood in the pulmonary vasculature resulted in a constant $64 \pm 5\%$ reduction in tissue damping (G) and a $55 \pm 4\%$ reduction in tissue elastance (H). This nearly-constant contribution of blood to lung hysteresivity was only reduced by positive end-expiratory pressure (PEEP). To identify a distinct structural subset of vessels in the lung potentially contributing to these observations, vascular casting and scanning electron microscopy of the lung demonstrated morphologically distinct vascular rings at the alveolar opening. Our results suggest that intravascular blood distension, likely attributable to a subset of vessels in the alveolar entrance ring, contributes a measurable scaffolding effect to the functional recruitment of the peripheral lung.

Introduction

A structural contribution of blood vessels to alveolar opening was first proposed in the late 19th century. Basch, in a series of experiments using pressure-limited bellows to insufflate open-chest dogs, demonstrated that vascular distension was associated with increased end-expiratory lung volumes [1, 2]—an effect he attributed to “lungengerüst” or the scaffolding effect of distended lung capillaries.

More recent studies have suggested that pulmonary mechanics are influenced by blood vessel distension as well as blood composition. Frank and co-workers showed that vascular distension increased lung volumes at a given applied pressure [3]. Gianelli et al. used the word “scaffolding” to describe the effect of perfusion on lung mechanics in an isolated dog heart-lung preparation [4]. In addition to confirming the improved compliance at mid-lung volumes, Giannelli et al. noted that the vascular effect was observed irrespective of flow; the scaffolding effect was observed with both static distension and active perfusion. Several workers have also suggested that “capillary erection” is essential in maintaining lung expansion after birth [5, 6].

Pulmonary mechanics are also influenced by pulmonary blood composition [7, 8]. The blood hematocrit can influence pulmonary vascular resistance [9] as well as the viscoelastic properties of the lung [10]. Petak and colleagues have recently shown that the viscous and elastic properties of the lung varied with hematocrit, suggesting the significant contribution of blood cells to lung viscoelasticity [11].

Although a contribution of blood distension to pulmonary mechanics is a consistent finding, specific structural correlates to these observations remain elusive. In this report, we examined the contribution of blood vessel distension to lung mechanics in normal mice and mice after varying degrees of parenchymal resection. Our studies suggest that a subset of blood vessels, likely those associated with the alveolar entrance ring, provide structural support for alveolar opening in the normal lung.

Methods

Mice.

8 to 12 week old C57/B6 mice (Jackson Laboratory, Bar Harbor, Maine), 22 to 30gm, were used in all experiments. The care of the animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, Md) with the protocol reviewed and approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Anesthesia and intubation.

The animals were anesthetized with an intraperitoneal injection of ketamine 100 mg/kg (Fort Dodge Animal Health, Fort Dodge, Iowa) and Xylazine 6 mg/kg (Phoenix Scientific, Inc., St. Joseph, MO). The glottis was directly visualized and intubated with a 18 gauge Angiocatheter (forced oscillation measurements)(BD Insyte, Sandy, Utah) and transferred to a FlexiVent rodent ventilator (Scireq, Montreal, QC Canada) as previously described [12].

Ventilator rates of 200/minute, tidal volume of 10 ml/kg with positive end-expiratory pressures of 3 cmH₂O and a pressure limit of 30 cmH₂O were used for the pneumonectomy procedure.

Pulmonary resection.

Mice undergoing surgical resection had constant heart rate and oximetry monitoring [13]. In mice undergoing surgical resection, the anterior chestwall was removed and lobar exclusion performed by clamp occlusion of the lobar bronchus and corresponding arterial blood supply.

Pulmonary mechanics.

Prior to all measurements, the pressure transducers and ventilator tubing of the FlexiVent (SciReq, Montreal, QC) were calibrated as described [13]. After intubation, mice were transferred to the FlexiVent system (SciReq) for pulmonary mechanics studies. All measurements were made in non-survival operations. Pulmonary mechanics were measured prior to, and immediately following, acute exsanguination. The animals were allowed to acclimate to the ventilator for two minutes before standardization of the volume history with 3 consecutive recruitment maneuvers ("TLC" by SciReq). Two maneuvers were performed. First, quasi-static pressure-volume loops were created by an 8-second steady inflation ramp to 30 cmH₂O, starting from a PEEP of 3 cmH₂O, with a 8-second passive deflation. Second, ventilation was briefly stopped and the animal passively exhaled to FRC; an 8 second multifrequency (0.5–19.5 Hz) oscillatory signal (Prime-8 by SciReq) was delivered with ventilation resumed at the completion. After measurements with a circulating blood volume, and the reassessment of deep anesthesia to minimize exsanguination-related cytokine influences [14], a venotomy was performed at the cavoatrial junction. Ongoing cardiac function resulted in exsanguination as reflected by a rapid change in lung color. When the lungs reached a uniform white appearance, forced oscillations measurements were repeated in the exsanguinated condition.

Input impedance model.

Respiratory system impedance (Z_{rs}) includes the components representing the lung (Z_L) and chest wall (Z_w) [15]. To eliminate the chestwall component of impedance, the anterior chestwall was removed for terminal measurements. The constant phase model [16] was fit to the input impedance to derive Newtonian resistance (R_n), tissue damping (G), tissue elastance (H) and hysteresivity (η) as described below:

$$Z_{rs} = Z_{aw} + \left(G - j H \right) / \omega^\alpha \quad \text{where } \alpha = (2/\pi) \tan^{-1}(1/\eta) \text{ and } \eta = G/H$$

A minimum of three measures were taken per animal, with measurements accepted at a minimum coherence of 0.89; consistently high coherence suggested input impedance with a low signal to noise ratio.

Corrosion casting and scanning electron microscopy.

The lung vessels were cannulated and perfused with 15–20ml of 37°C saline followed by a buffered 2.5% glutaraldehyde solution (Sigma) at pH 7.40. After casting of the microcirculation with 15 ml of PU4ii (SPI, West Chester, PA) diluted with 20% methylmethacrylate monomers (Aldrich) and caustic digestion, the lung corrosion casts were imaged after coating with gold in an argon atmosphere with a Philips ESEM XL30 scanning electron microscope. The images of the corrosion casts were obtained in regions demonstrating filling of the whole alveolar capillary bed from artery to vein without evidence of extravasation or pressure distension.

Light and transmission electron microscopy.

Lungs designated for microscopy were harvested after cannulation of the trachea. The tissue was fixed by instillation of 2.5 % buffered glutaraldehyde into the bronchial system followed by the instillation of 50% O.C.T. Tissue-Tek (Fisher) in saline. Post-fixation samples were harvested and processed according to standard protocols and embedded in Epon (Serva, Heidelberg, Germany). Tissue sections (5 µm) were stained elastic van Gieson (EvG) or Sirius red (Sigma) and analysed with a Zeiss Axiophot microscope (Zeiss, Oberkochen, Germany). 700 Å ultrathin sections were analysed using a Leo 906 digital transmission electron microscope (Leo, Oberkochen, Germany).

Fractional contribution of blood.

The contribution of blood to pulmonary mechanics was referred to as “fractional contribution of blood” (FCOB). FCOB was defined as

$$FCOB = 1 - \left(X_{blood} / X_{exsanguinated} \right)$$

Where X represents lung impedance measures of G and H.

Statistical analysis.

Data analysis was performed using XLstat (Addinsoft, USA, New York, NY) add-in for Microsoft Excel. Results were reported as mean $\pm$ one standard deviation unless otherwise noted. Statistical significance was determined using analysis of variance (ANOVA) with a p-value of 0.05. Pairwise comparisons were performed as needed using Tukey’s test. We used Pearson correlation coefficients to test the relationships between continuous variables. The significance level for the sample distribution was defined as $P < .05$.

Results

Pressure-volume relationship.

To define the functional contribution of intravascular blood volume to pulmonary function, the PV loops in open-chest control mice (normal blood volume) were compared to mice without a circulating blood volume (acutely exsanguinated)(Figure 1A). For any given inflation volume, the tracheal pressure in the exsanguinated mouse exceeded the tracheal pressure in the control condition (Figure 1B); the mean tracheal pressure difference was

most notable at mid- to high-inflation volumes (Figure 1C). Peak pressures at the higher lung volumes (>0.5 ml) were followed by a rapid decline in tracheal pressure—an apparent stress relaxation of the lung. When normalized to peak pressure, the greatest decline in tracheal pressure was observed at the highest lung volumes (Figure 1D, asterisks). To separate the peripheral and central mechanical effects of blood volume, multi-frequency lung impedance data was fitted to the constant-phase model [16]. The presence or absence of blood had no effect on central airway resistance (Figure 2A). In contrast, measures of tissue damping (G), tissue elastance (H) and hysteresivity (η) demonstrated a significant increase in exsanguinated mice relative to control mice (Figure 2B-D).

Effect of supra-normal perfusion.

To determine the effect of supra-normal blood perfusion of the lung, we studied the effect of varying degrees of parenchymal resection on pulmonary mechanics. With heart rate monitoring used as a relative surrogate of cardiac index [17], surgical resection proportionately increased the circulating blood flow through the remaining lung. In mice with or without blood, there was a progressive increase in G and H (Figure 3, closed circles).

Although the absolute difference in G and H between the conditions increased with the amount of parenchymal resection, the proportional contribution of blood—here, referred to as the fractional contribution of blood (FCOB)—was identical in all conditions. Regardless of the amount of remaining lung parenchyma and the concomitant increase in blood flow, the presence of blood in the pulmonary vasculature resulted in a constant $64\pm 5\%$ reduction in tissue damping (G) and a $55\pm 4\%$ reduction in tissue elastance (H). The constant fractional contribution of the blood to both dissipative (G) and elastic (H) processes was apparent when plotted as a function of the amount of lung resected (Figure 4A-B).

Assuming blood flow contributed to alveolar opening, we predicted that alternative mechanisms of recruiting lung units, such as PEEP, would decrease the apparent contribution of blood to measures of G and H. In mice with 2 normal lungs, a progressive increase in PEEP resulted in a progressive decrease in the fractional contribution of blood to measures of both dissipative (G) and elastic (H) processes (Figure 4C-D). These data suggested that the effects of PEEP and pulmonary blood flow were complementary mechanisms contributing to alveolar opening.

Vascular scaffold.

To identify the structural correlates to these functional observations, histologic analysis and corrosion casting of the microcirculation were performed. Despite the expected shrinkage of the corrosion casts, there was a consistent relationship between capillary and alveolar diameter; the capillary diameter was consistently 10% of the alveolar diameter (capillaries $3.59\pm 1.47\mu\text{m}$; alveoli $33.62\pm 10.18\mu\text{m}$) (Figure 5). Although it is likely that all peripheral capillaries contribute to a scaffold effect, the capillaries associated with the elastic line element are most likely to participate in alveolar opening [18]. The elastic line element maintains alveolar structure by balancing the distortion created by alveolar surface tension [19]. In histologic sections stained with Evg, capillaries were consistently identified at the tips of alveolar septa near the elastic line element (Figure 6A-B). TEM demonstrated

pulmonary capillaries intimately associated with the elastin fibers and collagen bundles characteristic of the elastin line element (Figure 6C-D).

Corrosion casting and scanning electron microscopy (SEM) was used to investigate the vessels associated with the elastin line element. SEM demonstrated circularly arranged blood vessels that defined the alveolar opening (Figure 7, red lines); these vessels appeared to define the alveolar entrance ring (AER). Notably, the AER vessels maintained continuity with neighboring vessels. Occasionally, a second “tier” of AER vessels were observed (Figure 7C, arrow). Note the cylindrical morphology of the AER vessels compared to the flattened morphology of most alveolar capillaries.

Discussion

Previous work has provided functional support for the vascular scaffold within peripheral lung units. Borst et al. localized the vascular effect to the alveolar capillaries or pulmonary veins by observing that lung compliance changed with increased left atrial pressures, but did not change over a wide range of pulmonary artery pressures [20]. Similarly, the rapid reversibility of the scaffold effect [4, 20] suggested that the blood volume contribution was related to structural distension of alveolar capillaries and not an indirect effect on tissue composition or surfactant function. In the present study, we provided additional functional evidence, based on lung impedance studies, for the scaffold effect of pulmonary blood flow. We also described the structural features of the blood vessels defining the AER. The unique structural features of the AER suggest that these vessels prominently contribute to the scaffold effect of pulmonary blood flow.

The functional evidence of the scaffold effect of blood capillary distension was based on multifrequency impedance measures of lung’s hysteretic properties (G and H). A fundamental observation in lung mechanics has been the nearly-invariant coupling of energy dissipative and elastic processes [21]; a constancy that has led to the structural damping hypothesis [22]. In our study, we used multi-frequency lung impedance data (FlexiVent) fitted to the constant phase model [16] to estimate the relative contribution of blood to these elemental processes. A striking finding was the nearly-invariant proportional contribution of blood to measures of G and H irrespective of the amount of lung tissue removed or the relative amount of pulmonary blood flow. These observations suggest that the increased impedance of the non-perfused lungs was a result of incomplete recruitment of alveoli—likely the result of the absence of the scaffolding effect of blood-filled capillaries.

To identify the anatomic location of the blood vessels potentially linked to alveolar opening and stress-bearing structures [19, 23], we examined corrosion casts of murine lungs. In the alveolar entrance, we observed vessels structurally distinct from alveolar capillaries within the alveolus. The capillaries were characterized by 1) circumferential continuity throughout the alveolar ring, 2) a cylindrical morphology when examined by corrosion casting and SEM, and 3) connectivity with other AER blood vessels. Perhaps an extension of directly perfused airway blood vessels [24], the AER vessels were notable in all conditions; in some cases, AER vessels were accompanied by a second “tier” of circumferential vessels. Although the structural features of AER blood vessels were distinctive, TEM demonstrated

that endothelial morphology was similar in both AER vessels and alveolar capillaries—a finding suggesting that the vessels are unique because of their location and circulatory relationships rather than any particular feature of endothelial cell biology.

A contribution of this study was the comparison of absent, normal and supra-normal blood perfusion to the mechanical properties of the lung. The limitations of this study include the difficulty in isolating the interdependent, interactive and adaptive elements contributing to the macro-level properties of the lung [25]. The complex spatial distribution of blood flow [26] and the viscoelasticity of the blood [11] are examples of two factors that deserve additional investigation. Further, the acute hemorrhage in these studies is associated with the release of endogenous mediators that influence lung mechanics [27, 28]. Although the anesthetic technique and the method of exsanguination were designed to minimize these influences, future studies of the pulmonary effects of hemorrhagic shock are likely to be relevant to our findings.

Our data indicated that the dominant positive effect of blood volume on lung mechanics was observed at low blood volumes; that is, the scaffolding effects were most profound between no blood and a normal blood volume. There was no significant change in the contribution of blood between a normal blood volume and a near-doubling of blood flow through the lungs (post-surgical resection). These observations suggested that the mechanical benefit of blood distension are perhaps more relevant to the mechanism of lung expansion at birth [5, 6] or during lung growth [29, 30], than the acute [31, 32] or chronic [33] congestion associated with heart failure or longstanding congenital heart disease. Although the scaffolding effect of capillary distension is controversial [34], the changes in vascular resistance observed in newborns [35] are potentially compatible with a structural contribution to the AER postulated here.

The interaction and interdependence of blood and gas volume in the lung likely reflects a practical evolutionary efficiency. Blood has structural properties ranging from the incompressibility of plasma [36] to the viscoelasticity of red blood cells [37]. Our data suggests that these properties are utilized to provide structural support for terminal respiratory units. Blood is also central to the primary function of the lung; namely, gas exchange. The structural interdependence of blood and gas volume in the lung suggests that the opening of terminal respiratory units in the presence of blood volume may contribute to such basic functional processes as the matching of ventilation and perfusion.

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Abbreviations:

AER	alveolar entrance ring
Evg	elastic van Gieson
FCOB	fractional contribution of blood

G	tissue damping
H	tissue elastance
η	hysteresivity
IP	intrapertitoneal
P	pressure
PEEP	positive end-expiratory pressure
R_n	Newtonian resistance
SA	surface area
SD	standard deviation
V	volume
Z_{rs}	respiratory system impedance
Z_L	lung impedance
Z_w	chest wall impedance

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Figure 1.

The contribution of vascular distension on quasi-static pressure-volume measurements in normal lungs. A) Pressure-volume loop of an exsanguinated mouse (black line) and a mouse with a normal circulating blood volume (control, gray line) obtained with 0.1 ml volume increments; representative (modal) tracings of $N = 7$ mice are shown. B) Pressure-time plot with similar 0.1ml volume and 1 second time increments. C) Mean quasi-static tracheal pressure difference () between an exsanguinated and a control mouse on lung inflation (solid circles) and deflation (open circles). D) Apparent stress relaxation normalized to the fraction of peak tracheal pressures from the pressure-time plot (B). The 4 highest pressure curves from the exsanguinated mouse (black line) and 2 highest curves from the control mouse (gray line) are shown. The asterisks identify corresponding curves in panels B and D.

Figure 2.

Effect of blood volume on lung impedance measurements using the forced oscillation technique (FlexiVent). A) Newtonian resistance (R_n) showed no difference with or without blood volume. B,C) Measures of tissue damping (G) and tissue elastance (H) demonstrated significantly increased values in bloodless mice (**, $p < .001$). D) Hysteresivity, reflecting the relationship between energy dissipation and energy conservation in the tissue, was also significant (*, $p < .05$). Triplicate measures per mouse; each data point represents $N = 5$ mice.

Figure 3.

Effect of blood volume on lung impedance measurements in mice after varying amounts of lung parenchymal resection. Percent lung reduction corresponds to the resection of the middle lobe (5%), lower lobe (28%), cardiac lobe (6%) and upper lobe (19%); parenchymal volumes were mean CT lobar volumes calculated after a 30 cm H₂O recruitment maneuver in 3 mice. Newtonian resistance (R_n) showed no difference with or without blood volume at any percent lung reduction ($p > .05$). B,C) Measures of tissue damping (G) and tissue elastance (H) demonstrated a progressive increase with an increasing percentage of parenchymal resection in both conditions (*, $p < .01$). D) Hysteresivity was elevated in the exsanguination group, but showed no change with varying amounts of parenchymal resection. N = 5 mice per data point.

Figure 4.

Effect of lung reduction and PEEP on the fractional contribution of blood (FCOB) to lung impedance measurements. Despite varying amounts of parenchymal resection, the proportional effect of blood distension, here, referred to as “FCOB” (see Methods), on measures of tissue damping G (panel A, Pearson’s $r = 0.914$, $p = 0.084$) and tissue elastance H (panel B, Pearson’s $r = 0.916$, $p = 0.085$) were unchanged; a finding suggesting that the excess perfusion of the alveolar capillaries did not contribute to the stress-bearing element reflected by the constant phase model measures of G and H. In mice with 2 lungs, increasing levels of PEEP lowered the FCOB to G (panel C, Pearson’s $r = -0.898$, $p = 0.10$) and H (panel D, Pearson’s $r = -0.988$, $p = 0.01$) suggesting a complementary contribution to alveolar opening. Mean of $N = 5-7$ mice per data point is shown.

Figure 5.

Morphometric assessment of vascular corrosion casts. The corrosion casts of control adult mice ($N = 8$ mice) evaluated; 639 capillaries were assessed in 128 alveoli using tilt-angle scanning electron microscopy (SEM) [29]. Dotted lines reflect 95% confidence bands. The mean capillary diameters were 10.6% of the mean alveolar diameters.

Figure 6.

Histology of alveolar septa. A) Light microscopic image of a normal lung tissue section (5 μ m) stained with Evg. The section demonstrates septal "ends" [30] reflecting the alveolar opening in cross section (circles). Bar = 30 μ m. B) Higher resolution image demonstrates both the black elastin fibers (arrow) and the gold erythrocytes (double arrow) at the tip of the alveolar septa. Bar = 10 μ m. C-D) Transmission electron microscopy of septal "ends" of the alveolar opening demonstrating the structural relationship between collagen and elastin (el) fibers and septal capillaries (c). C, Bar = 3 μ m; D, Bar = 10 μ m.

Figure 7.

Corrosion casts and scanning electron microscopy of vascular rings at the alveolar opening. A-D) The alveolar entrance ring (AER) blood vessels defined the alveolar opening (red lines) while maintaining continuity with neighboring vessels. Occasionally, a second “tier” of AER vessels were observed (C, arrow). Note the cylindrical morphology of the AER vessels compared to the flattened morphology of most alveolar capillaries. A-D, bar = 20 μ m.