Preventing mediastinal shift after pneumonectomy impairs regenerative alveolar tissue growth

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Hsia, C. C. W., E. Y. Wu, E. Wagner, and E. R. Weibel. Preventing mediastinal shift after pneumonectomy impairs regenerative alveolar tissue growth. Am J Physiol Lung Cell Mol Physiol 281: L1279–L1287, 2001.—To examine the effects of mechanical lung strain on regenerative growth of alveolar septal tissue after pneumonectomy (PNX), we replaced the right lungs of adult dogs with a custom-shaped inflatable silicone prosthesis. The prosthesis was either inflated (Inf) to maintain the mediastinum at the midline or deflated to allow mediastinal shift. The animals were euthanized 15 mo later, and the lungs were fixed at a constant distending pressure. With the Inf prostheses, lung expansion, alveolar septal tissue volumes, surface areas, and diffusing capacity of the tissue-plasma barrier were significantly lower than with the deflated prostheses; the expected post-PNX tissue responses were impaired by 30–60%. Capillary blood volume was significantly higher with Inf prostheses, consistent with microvascular congestion. Measurements in the Inf group remained consistently and significantly higher than those expected for a normal left lung, indicating persistence of partial compensation. In one dog, delayed deflation of the prosthesis 9–10 mo after PNX led to vigorous lung expansion and septal tissue growth, particularly of type II epithelial cells. We conclude that mechanical lung strain is a major signal for regenerative lung growth; however, other signals are also implicated, accounting for a significant fraction of the compensatory response to PNX.

METHODS

Prosthesis for the Right Lung

These procedures have been described previously (33). The dog was anesthetized, intubated, and ventilated. In the supine position and with the breath held at functional residual capacity (FRC), consecutive transverse magnetic resonance images were obtained at 10-mm intervals from the apex to the costophrenic angle. The outline of the lung was digitized from each image, and the area was measured by software available on the scanner. Lung volume represented by each image was calculated from the product of the area and thickness, and the total lung volume was calculated from the summation of all images. A three-dimensional model of the right lung was reconstructed from stacked images. An inflatable silicone prosthesis (CUI, Carpinteria, CA) was fabricated in the exact shape and size of the reconstructed lung model, with an injection tube attached to the dorsolateral surface via a reinforced patch.

Animal Surgery and Implantation of Prosthesis

Protocols were approved by the Institutional Animal Care and Research Advisory Committee. Adult male foxhounds...
were studied beginning at 1 yr of age. Each dog underwent resection of the right lung (55% of total lung volume, perfusion, and diffusing capacity), under general anesthesia, following established procedures (10). After removal of the right lung, the silicone prosthesis was implanted into the empty right hemithorax. The injection tube was brought out through an intercostal space, tunneled to the nape of the neck, connected to a septal filling port, and buried subcutaneously. In half the animals (n = 5), the prosthesis was inflated with an equal mixture of air and SF6 to a volume 20% above the FRC of the animal measured in the supine position [inflated (Inf) group]. Mixing air with SF6 retards the rate of gas absorption from the prosthesis. In the other half of the animals (n = 5), the prosthesis remained deflated, containing <50 ml of gas to prevent pleating [deflated (Def) group].

After recovery, the volume of the prosthesis was checked by helium dilution via the subcutaneous filling port weekly for the first month and then at monthly intervals. After each measurement, the prosthesis was refilled to the desired level with the air–SF6 mixture, and the position of the mediastinum was verified by chest X ray. The rate of gas loss from the prosthesis was found to be ∼20–25%/mo.

Beginning 1 mo after surgery, animals were trained to run on a treadmill by protocols described previously (13). Physiological studies performed at rest and during exercise have been reported separately, along with radiological assessment by CT scan (33). Animals were euthanized 12–15 mo after PNX.

Euthanasia and Morphometric Analysis

The dog was deeply anesthetized with pentobarbital sodium (25 mg/kg IV) and intubated via a tracheostomy. The lung was collapsed through bilateral intercostal incisions and then reinfated within the thorax by the intratracheal instillation of 2.5% glutaraldehyde (buffered in 0.03 M potassium phosphate, pH 7.40, 350 mosM) at a constant hydrostatic pressure (25 cmH2O) above the highest point of the sternum in the supine position. An intravenous overdose of pentobarbital sodium was given simultaneously. After 60 min of fixation in situ, the lungs and heart were removed en bloc and immersed in 2.5% glutaraldehyde for at least 1 wk.

The volume of the intact lung was measured by two methods: 1) immersion displacement without release of the airway pressure and 2) the Cavalieri principle (7) after the pressure was released and the lung was sectioned. The left lung was divided into upper and lower strata. The upper stratum consisted of the upper lobe and lingula; the lower stratum consisted of the lower lobe. Each stratum was sectioned serially at 2-cm intervals, and each cut surface was photographed with 35-mm Ektachrome film. Lung volume after sectioning was estimated from the photographs with the Cavalieri principle (31). Diffusing capacities of the alveolar tissue–plasma barrier and the lung for oxygen were calculated by a modified version (32) of the model established by Weibel (30).

Data Analysis

Data were normalized for body weight and are expressed as means ± SE. Results were also compared with those previously reported by our laboratory (12) for normal adult dog lungs prepared and analyzed in the same way (sham; n = 5 dogs). Group comparison was by analysis of variance with post hoc comparison by Fisher’s and Student-Newman-Keuls multiple comparison methods with the use of a commercial software package (StatView, version 5.0, SAS Institute, Cary, NC). A P value < 0.05 was considered significant.

RESULTS

Mediastinal Shift

Dogs tolerated the prosthesis without complication, and pleuromediastinal mobility was not impaired. Illustrative CT images (Fig. 1) from these animals (33) show that with the Def prosthesis, there was gross lateral expansion of the left lung, displacing the mediastinum into the left hemithorax. With the Inf prosthesis, the mediastinum remained at the midline. One animal in the Inf group (dog Z) developed a leak in the prosthesis 9–10 mo after surgery, and the mediastinum could not be maintained at the midline thereafter. Volume of the remaining lung in this dog progressively increased over the subsequent month. Morphometric data from this dog have been excluded from statistical comparison and are shown separately, leaving four
dogs in the Inf group and five in the Def group. Physiological and radiological assessments in these animals were reported elsewhere (33).

**Gross Postmortem Examination**

At postmortem, there was no pleuromediastinal effusion or adhesion in any dog. There was thickening and granular tissue formation over the right parietal pleural and diaphragmatic surfaces that were in contact with the prosthesis. The extent of granular tissue formation over the right parietal surface was similar between groups, but over the diaphragmatic surface, granular tissue was more pronounced in the Inf group because of a greater contact area when the prosthesis was inflated. In the Def group, a small amount of gas (<200 ml) eventually accumulated within the prosthesis.

**Body Weight and Lung Volume**

Dogs with Inf prostheses had a significantly lower mean body weight than those with Def prostheses even before surgery; however, body weight did not change significantly after PNX in either group. Results are shown in Table 1. There was no difference in morphometric hematocrit between groups.

In the Def group, volume of the intact remaining left lung as measured by immersion displacement increased approximately twofold compared with a normal left lung, to within 90% of that in two lungs of normal dogs (12). Volume of the sectioned lung, estimated with the Cavalieri principle, was consistently lower than that of the intact lung, consistent with our laboratory’s previous observations in dog lungs (25), indicating that alveolar septa were unfolded or under strain at 25 cmH2O fixation pressure. In the Inf group, volume of the intact lung averaged 69% of that in the Def group (P = 0.02). Furthermore, lung volume did not change significantly after sectioning in the Inf group, suggesting that alveolar septa were under less strain at the same fixation pressure. Despite a lower body weight in dogs with inflated prosthesis, mass-specific lung volume after sectioning was similar in both groups. Volumes of the sectioned lungs did not differ between the two groups.

**Volume and Surface Densities of Septal Components**

Volume densities of coarse parenchyma and fine parenchyma per unit of lung volume were both significantly higher in the Inf than in the Def group, indicating crowding of parenchyma in the Inf group (Table 2; Fig. 2). The surface areas of alveoli and capillaries per volume of septum were smaller in the Inf group (by 23 and 20%; P = 0.01 and 0.0004, respectively). However, the volume density of capillaries per volume of septum was significantly higher in the Inf group than in the Def group (28%; P = 0.0002), indicating capillary congestion in the Inf group. Volume density of alveolar epithelium was significantly lower in the Inf than in the Def group due to a lower density of type I epithelium; the density of type II epithelium was slightly but not significantly lower in the Inf group. Volume densities of interstitium, endothelium, and extravascular alveolar tissue per volume of septum were all smaller in the Inf group compared with the Def group (by 29, 23, and 26%, respectively; P = 0.004–0.0002). Because volume densities of extravascular septal tissue and capillary blood changed in opposite directions, volume density of the entire septum per unit of lung volume was similar between groups.

Mean septal thickness was larger by 33% in the Inf group compared with the Def group (P = 0.01), essentially as a result of larger capillary volume. Harmonic mean thickness of the diffusion barrier from epithelial-air interface to the red cell membrane was similar between Inf and Def groups but 26% higher than that expected in normal lungs (P < 0.007) (12). A similar increase in arithmetic and harmonic mean septal thickness had also been observed previously in pneumonectomized adult dogs without prostheses (12).

**Absolute Volumes and Surface Areas of Septal Components**

Results are shown in Table 3 and are compared with results from adult sham control dogs in Figs. 3 and 4 (12). Comparing the Inf with the Def group, the volume of epithelium was significantly lower (31%; P = 0.008) due to a lower type I epithelial cell volume; volume of
Type II epithelium was slightly but not significantly lower. Volumes of interstitium and endothelium were 31 (P = 0.02) and 27% (P = 0.002) lower, respectively (Fig. 3A). Extravascular septal tissue volume was 30% lower (P < 0.003). However, capillary blood volume was 21% higher in the Inf group (P = 0.01; Fig. 3B). Because volumes of extravascular septal tissue and capillary blood changed in opposite directions, the total volume of the septum was not significantly different between the Inf and Def groups. Alveolar and capillary surface areas were reduced by 28 and 24%, respectively, in the Inf group than in the Def group (P < 0.002; Fig. 4). In both groups, septal cellular and capillary blood volumes as well as alveolar and capillary surface areas were consistently and significantly greater than those in a normal left lung (P = 0.0001–0.04).

**Morphometric Lung Diffusing Capacities**

Results are shown in Table 4 and are compared with results from adult sham control dogs (Fig. 5) (12). Erythrocyte diffusing capacity for oxygen was similar

<table>
<thead>
<tr>
<th>Relative volume and surface area</th>
<th>RPNX</th>
<th>Inf</th>
<th>Def</th>
<th>P Value</th>
<th>Dog Z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative volume</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Coarse parenchyma volume/lung volume</td>
<td>0.8788 ± 0.0181</td>
<td>0.8370 ± 0.0083</td>
<td>0.01</td>
<td>0.884</td>
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<tr>
<td>Fine parenchyma volume/lung volume</td>
<td>0.8042 ± 0.0186</td>
<td>0.7692 ± 0.0092</td>
<td>0.05</td>
<td>0.821</td>
<td></td>
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<tr>
<td>Septum volume/lung volume</td>
<td>0.1187 ± 0.0162</td>
<td>0.1188 ± 0.0080</td>
<td>NS</td>
<td>0.078</td>
<td></td>
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<tr>
<td>Type I epithelium volume/septum volume</td>
<td>0.0750 ± 0.0018</td>
<td>0.1035 ± 0.0086</td>
<td>0.03</td>
<td>0.092</td>
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<td>Type II epithelium volume/septum volume</td>
<td>0.0365 ± 0.0077</td>
<td>0.0468 ± 0.0077</td>
<td>NS</td>
<td>0.061</td>
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<tr>
<td>Total epithelium volume/septum volume</td>
<td>0.1115 ± 0.0067</td>
<td>0.1502 ± 0.0103</td>
<td>0.04</td>
<td>0.154</td>
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<tr>
<td>Interstitium volume/septum volume</td>
<td>0.1816 ± 0.0219</td>
<td>0.2525 ± 0.0169</td>
<td>0.004</td>
<td>0.192</td>
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<td>Endothelium volume/septum volume</td>
<td>0.0846 ± 0.0030</td>
<td>0.1104 ± 0.0034</td>
<td>0.0030</td>
<td>0.100</td>
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<td>Extravascular tissue volume/septum volume</td>
<td>0.3777 ± 0.0161</td>
<td>0.5138 ± 0.0189</td>
<td>0.0002</td>
<td>0.446</td>
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<td>Capillary blood volume/septum volume</td>
<td>0.6223 ± 0.0161</td>
<td>0.4862 ± 0.0189</td>
<td>0.0002</td>
<td>0.554</td>
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<tr>
<td><strong>Relative surface area, cm⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alveolar surface area/septum volume</td>
<td>3,355 ± 296</td>
<td>4,378 ± 176</td>
<td>0.01</td>
<td>3,744</td>
<td></td>
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<tr>
<td>Capillary surface area/septum volume</td>
<td>3,316 ± 164</td>
<td>4,145 ± 135</td>
<td>0.004</td>
<td>4,098</td>
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<tr>
<td>Septal thickness, μm</td>
<td>6.16 ± 0.64</td>
<td>4.63 ± 0.17</td>
<td>0.01</td>
<td>5.35</td>
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<tr>
<td>Harmonic mean barrier thickness, μm</td>
<td>0.99 ± 0.03</td>
<td>0.98 ± 0.06</td>
<td>NS</td>
<td>0.87</td>
<td></td>
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Values are means ± SE. P values are for Inf vs. Def.

Fig. 2. Micrographs of lung parenchyma from dogs with inflated and deflated prostheses. Thickened septum and high capillary density within septum are evident in the dog with inflated prosthesis. Original magnification, ×95.
between Inf and Def groups; both were significantly higher than in the left lung of sham control animals \((P < 0.001)\). In the Def group, diffusing capacity of the tissue-plasma barrier was 61\% higher than in sham control lungs \((P < 0.0004)\). In the Inf group, the diffusing capacity of the tissue-plasma barrier was 28\% lower \((P = 0.006)\) than in the Def group and not significantly different from that in sham control lungs \((P > 0.05)\). Overall lung diffusing capacity was 16\% lower in the Inf than the Def group \((P < 0.04)\); lung diffusing capacity in both groups remained significantly higher than in the sham control lungs \((P < 0.003)\).

**Effect of Delayed Lung Expansion After PNX**

In dog Z, in which the inflated prosthesis became deflated unexpectedly 9–10 mo after PNX, there was a progressive mediastinal shift and increase in volume of the left lung over the subsequent 3 mo as measured by a rebreathing technique at rest and during exercise \((33)\). Comparison of morphometric data from this dog 13 mo after PNX with those from the Inf group showed that lung volume was 74\% larger and septal volume density 34\% lower in this dog. Volumes and surface areas of septal tissue compartments per unit of lung were consistently higher than the mean of the Inf group. In particular, the volume of type II epithelium per unit volume of septum was 67 and 30\% higher than the Inf and Def group means, respectively, consistent with vigorous epithelial cell growth occurring after delayed prosthesis deflation. Volume density of capillaries was intermediate between Inf and Def group means, consistent with relief of microvascular congestion after delayed prosthesis deflation. A similarly consistent pattern was seen in the absolute volumes and surface areas of all septal components as well as in lung diffusing capacities (see Tables 1–5).

**DISCUSSION**

**Critique of Methods**

The presence of intrathoracic granular tissue indicates a local friction-induced inflammatory reaction that did not affect the weight, exercise performance, or clinical well-being of the animals. The inflation volume of the prosthesis was 20\% above the resting FRC of a normal right lung, resulting in a mean lung diffusing capacity volume between refills of \(\sim 10\%\) above resting supine FRC; this volume is below the FRC of a conscious prone animal. In addition, the post-PNX increase in tidal volume per unit of remaining lung was not completely prevented. We cannot rule out a growth-stimulating effect due to cyclical lung stretch. However, in adult dogs studied previously before and after left PNX (45\% resection), there was no compensatory lung growth despite a 90\% increase in tidal volume borne by one lung \((11)\), suggesting that tidal lung stretch must increase at least twofold before lung growth is induced. With the prosthesis inflated, the increase in lung volume during exercise never exceeded 30\% \((33)\). We did not implant the prosthesis in normal dogs without PNX; hence, we cannot rule out a silicone-induced nonspecific tissue response; this possibility does not invalidate the comparison between the Inf and Def groups because the same prosthesis was implanted in both groups.

**Mechanical Signals for Post-PNX Lung Growth**

Mechanical effects of PNX include volume expansion and increased perfusion to the remaining lung. The resulting alveolar strain and endothelial distension and shear are believed to induce proliferation of septal cells \((21)\). Mechanical strain induces in vitro proliferation of alveolar epithelial cells \((14)\), stimulates glycosaminoglycan and proteoglycan exocytosis \((34)\), and increases gene expression of surfactant-associated proteins \((23)\). Accelerated fetal lung growth after tracheal ligation in utero has been ascribed to mechanical effects of increased intratracheal pressure. Replacing tracheal fluid with saline inhibits lung hypertrophy after tracheal ligation in fetal lambs \((20)\), suggesting the presence of tracheal fluid growth factors induced by lung distension. Sustained segmental lung distension with perfluorocarbon in neonatal lambs also accelerates lung growth in an age-dependent fashion \((17)\).
Weanling ferrets subjected to continuous positive airway pressure for 2 wk demonstrate increased total lung capacity, weight, protein, and DNA content without any change in lung recoil, suggesting strain-induced parenchymal remodeling (35).

An early study by Cohn (5) reported that preventing post-PNX lung expansion eliminates compensatory lung growth. However, Fisher and Simnett (6) measured the mitotic index in post-PNX rat lungs and found that plombage delayed but did not eliminate cellular proliferation. Brody et al. (2) reached the same conclusion by measuring DNA synthesis in pneumonectomized mice. Olson and Hoffman (19) found that wax plombage did not completely prevent expansion of the remaining lung in pneumonectomized rabbits. Instead of expanding laterally across the midline, the remaining lung changed shape and elongated in the cranial-caudal direction. These data implicate stimuli other than lung strain during compensatory lung growth, although alveolar structure was not specifically studied previously.

**Effects of Preventing Mediastinal Shift After PNX**

**Lung volume.** A positive intrapulmonary pressure during fixation expands the lung, unfolds alveolar septa, and imposes strain on septal tissue. The fixed septa are not rigid but possess residual elasticity (18) and tend to refold on subsequent sectioning when pressure is released. In this study, we found that lung volume decreased in animals with deflated prostheses after airway pressure was released, consistent with observations in previous studies by our laboratory (25). The fixed volume and shape of the inflated prosthesis may limit the way the thorax can respond to a positive airway pressure and thus lower the effective compliance of the rib cage. If so, at a given distending pressure, alveolar septa would be less unfolded, septal strain would be lower, and less volume change would occur on release of the distending pressure. It is, however, important to note that this observation is of no consequence for the morphometric comparison because all histological analysis was related to lung volumes measured with the Cavalieri principle after sectioning, i.e., without pressure.

In the Inf group, volume of the intact left lung was reduced, the left diaphragm was at a lower station, and the right hemithorax bulged out compared with the Def group (33). These observations indicate that prosthesis inflation successfully prevented mediastinal shift and altered the shape of the left lung. Preventing mediastinal shift did not eliminate the compensatory increase in lung volume. Volume of the intact lung in the Inf

![Fig. 3. A: epithelial, interstitial, and endothelial cell volumes are lower in dogs with inflated (Inf) compared with deflated (Def) prostheses. R and L, right and left lungs, respectively, of sham control animals. B: in the Inf prosthesis group, extravascular septal tissue volume is decreased and capillary blood volume is increased compared with DEF prosthesis group; hence, total septal volume is not different between these 2 groups. Sham data are from adult dogs studied previously with the same methods (12). Values are means ± SE. Significant difference (P < 0.05) compared with: *sham left lung; †sham both lungs; †Def group.](http://ajplung.physiology.org/issue◥)

![Fig. 4. Alveolar and capillary surface areas are lower in dogs with Inf compared with Def prosthesis. Values are means ± SE. In both prosthesis groups, surface areas are significantly higher than in the left lungs of adult sham control animals studied previously (12). Significant difference (P < 0.05) compared with: *sham left lung; †sham both lungs; †Def group.](http://ajplung.physiology.org/issue◥)
group was still significantly larger than that of a normal left lung (12). In fact, lung volume measured after sectioning was similar regardless of whether the prosthesis was inflated or deflated. Physiological and radiological assessments in the Inf group show that lateral bulging of the left hemithorax and depression of the left hemidiaphragm contributed to a 20% increase in lung volume above normal (33). Thus post-PNX lung expansion is not simply a passive response to a negative intrathoracic pressure to fill an empty space. There appear to be other factors that compel the remaining lung to expand even when lung strain is relieved and when space is not readily available.

**Tissue volume and surface area.** The septal tissue layer, composed of epithelium, interstitium, and endothelium, represents the structural backbone bounded by the alveolar and capillary surfaces, whereas capillary blood volume is a variable component that also depends on hemodynamic conditions. The extravascular tissue volume was 45% larger in the Def than in the Inf group; the alveolar and capillary surfaces that bound the tissue layer were similarly larger, by 39 and 32%, respectively. As a result, the volume-to-surface ratio of the tissue layer (an estimate of the mean thickness of the tissue layer) was similar in both groups. Also, the composition of the tissue layer was identical in both groups: 30% epithelium, 48% interstitium, and 22% endothelium (Table 5); this corresponds to normal dog lung structure and is similar to that in pneumonecтомized adult dogs without a prosthesis that were studied previously (12).

In the Def group, total septal tissue volume as well as alveolar surface area increased to approximately the values in both (right and left) sham control lungs, i.e., 99% of total volume and 88% of total alveolar surface. To achieve this, the alveolar surface and tissue volume had to increase by factors of 1.8 and 2.2, respectively. In the Inf group, the alveolar surface and tissue volume of the residual left lung still enlarged 1.3 and 1.5 times, respectively, compared with the normal left lung. The ratio of tissue volume to alveolar surface and the relative proportions of cellular constituents within the septum were unchanged from normal values, suggesting persistent compensatory growth of all septal tissue constituents even in the absence of post-PNX lung strain.

**Capillary volume.** Capillary volume was 30% larger in the Inf than in the Def group, resulting in a 60% higher capillary volume per alveolar surface (i.e., capillary loading). Capillary surface area was not increased but showed the same relationship to alveolar surface area in both groups. Thus capillaries in the Inf group were congested because capillary loading of the alveolar septa was ~38% higher than that in control dog lungs. In the Def group, capillary loading was ~20% smaller than in sham control lungs. Capillary volume is a dynamic parameter strongly affected by hemodynamic conditions. Capillary congestion could have occurred in the Inf group simply because a given cardiac output must be accommodated within a smaller microvascular bed. Alternatively, the inflated prosthesis may have mechanically restricted pulmonary venous outflow, perhaps by removing the distending force on alveolar corner vessels normally associated with lung expansion.

**Morphometric diffusing capacities.** The lower diffusing capacity of the lung for oxygen and of the alveolar tissue-plasma barrier as estimated by morphometry in animals with inflated prostheses correlated directly with a significantly reduced membrane and lung diffusing capacity for carbon monoxide (33), an elevated alveolar-arterial oxygen tension gradient, and a lower

<table>
<thead>
<tr>
<th>RPNX</th>
<th>Inf</th>
<th>Def</th>
<th>P Value</th>
<th>Dog Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{O_{2}}$, ml·min⁻¹·s⁻¹·mmHg⁻¹</td>
<td>0.061 ± 0.004</td>
<td>0.067 ± 0.003</td>
<td>NS</td>
<td>0.065</td>
</tr>
<tr>
<td>$D_{E_{O_{2}}}$, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>10.71 ± 0.75</td>
<td>9.73 ± 0.89</td>
<td>NS</td>
<td>11.62</td>
</tr>
<tr>
<td>$D_{bO_{2}}$, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>5.26 ± 0.33</td>
<td>7.28 ± 0.58</td>
<td>0.006</td>
<td>7.95</td>
</tr>
<tr>
<td>$D_{tO_{2}}$, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>3.48 ± 0.06</td>
<td>4.11 ± 0.29</td>
<td>0.04</td>
<td>4.70</td>
</tr>
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</table>

Values are means ± SE normalized by body weight (kg). $Q_{O_{2}}$, rate of $O_{2}$ uptake by whole blood; $D_{E_{O_{2}}}$, diffusing capacity of erythrocytes; $D_{bO_{2}}$, diffusing capacity of tissue-plasma barrier; $D_{tO_{2}}$, lung diffusing capacity. $P$ values are for Inf vs. Def.
lung diffusing capacity for oxygen as measured by the multiple inert gas elimination technique (Johnson RL Jr., Hsia CCW, Wu EY, Estrera AS, Wagner H, and Wagner PD, unpublished observations) at a given exercise workload. The consistency among these independent measures of gas exchange underscores the concordance of structure-function relationships as well as the validity of the morphometric model of diffusing capacity. Gas-exchange impairment associated with the inflated prosthesis was clearly due to the lower alveolar-capillary surface area, whereas capillary congestion had only a mild effect on lung diffusing capacity for oxygen. Our cumulative data directly comparing morphometric and physiological estimates of diffusing capacity in the same animals (25) provide strong evidence that morphometric estimates closely reflect the physiological reserves for diffusive gas transport at peak exercise.

Effects of Delayed Mediastinal Shift

McBride (15) replaced the resected lung in ferrets with a silicone balloon filled with mineral oil. Thirteen weeks later, the remaining lung was still capable of expanding when oil was removed from the balloon. Our data from dog Z demonstrate that the remaining lung is capable of responding to isolated lung stretch 9–10 mo after PNX. After delayed prosthesis deflation, lung volume, exercise capacity, and physiological lung diffusing capacity increased progressively (33). Septal cellular response was global and vigorous, leading to higher volumes and surface areas of all septal tissue compartments relative to dogs with continuously inflated prostheses and approaching the mean values of the Def group. In particular, volume of type II epithelial cells was twice as high as in the Inf group, even exceeding that in the Def group by 38%. It is believed that type II cells are progenitors of type I cells, and proliferation of type II cells eventually increases epithelial cell volume and surface area as well as diffusing capacity. The response pattern of dog Z after delayed lung stretch is in keeping with this sequence of events. Although results from one animal cannot be conclusive, they illustrate two key points. 1) Delayed prosthesis deflation is a feasible method for isolating the effects of mechanical lung strain after other perturbations associated with PNX (increased perfusion, distortion of thorax and respiratory muscles, tissue trauma, and wound healing) have stabilized. 2) The potential for initiating a compensatory response is preserved for at least 9–10 mo. The signal inducing compensation need not be present at the time of PNX but can be imposed later without apparently affecting the magnitude of the compensatory response. These preliminary observations need to be corroborated in future studies.

Other Signals for Regenerative Lung Growth

After PNX, pulmonary perfusion per lung unit effectively doubles. With the prosthesis inflated, the same cardiac output must flow through a smaller lung, leading to greater endothelial distension and shear. Capillary congestion in the Inf group was evidenced by a higher capillary blood volume measured during exercise (33) as well as postmortem. In the Inf group, pulmonary arterial pressure was higher at a given cardiac output relative to that in the Def group (Wu EY, Hsia CCW, and Johnson RL Jr., unpublished data); the higher pressure may have imposed an additional stimulus for capillary growth. Haworth et al. (8) found that increasing pulmonary perfusion in newborn pigs accelerated alveolar proliferation in the contralateral lung. However, McBride et al. (16) showed that restricting pulmonary perfusion by banding one lobar pulmonary artery in ferrets had no effect on post-PNX weight, volume, protein, and DNA content of either the banded or the unbanded remaining lobes. Bronchial blood flow was not controlled in these previous studies (8, 16), and the role of endothelial distension and shear in compensatory lung growth remains to be defined.

In summary, we conclude that preventing post-PNX mediastinal shift successfully restricts mechanical lung strain at a given distending pressure. However, compensatory increase of lung volume is not eliminated; only expansion across the midline is prevented, and the remaining lung continues to enlarge in other

<table>
<thead>
<tr>
<th>Volume-to-volume ratio</th>
<th>Inf</th>
<th>Def</th>
<th>Without prosthesis</th>
<th>Dog Z</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I epithelium/septal tissue</td>
<td>0.20 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.22 ± 0.00</td>
<td>0.21</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Type II epithelium/septal tissue</td>
<td>0.10 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.14</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Total epithelium/septal tissue</td>
<td>0.30 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.32 ± 0.00</td>
<td>0.35</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Interstitium/septal tissue</td>
<td>0.48 ± 0.04</td>
<td>0.49 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>0.43</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Endothelium/septal tissue</td>
<td>0.23 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.22</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Capillary blood/septal tissue</td>
<td>1.67 ± 0.11†‡</td>
<td>0.96 ± 0.07</td>
<td>1.10 ± 0.03</td>
<td>1.25</td>
<td>1.35 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume-to-surface ratio</th>
<th>Inf</th>
<th>Def</th>
<th>Without prosthesis</th>
<th>Dog Z</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septal tissue/alveolar surface</td>
<td>1.17 ± 0.18</td>
<td>1.19 ± 0.08</td>
<td>1.17 ± 0.05</td>
<td>1.19</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>Capillary blood/alveolar surface</td>
<td>1.90 ± 0.14*‡</td>
<td>1.12 ± 0.05</td>
<td>1.29 ± 0.05</td>
<td>1.48</td>
<td>1.39 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. Significantly different (P < 0.05) compared with: *sham; †RPNX-Def group; ‡RPNX group (adult dogs after RPNX without prosthesis). Sham and RPNX without prosthesis data are from Hsia et al. (12). Data from dog Z were excluded from statistical comparison.
directions. Preventing lung strain is associated with microvascular congestion, septal crowding, and impaired septal tissue growth leading to lower lung diffusing capacities. Nevertheless, septal tissue volumes and surface areas in dogs with inflated prostheses remain significantly above those expected for normal left lungs, implicating other signals for lung growth apart from mechanical strain. In one dog, delayed deflation of the prosthesis was followed by progressive lung expansion, improved exercise lung diffusing capacity, and vigorous cellular growth, particularly of type II epithelial cells, suggesting that cellular response to isolated lung stretch is preserved for 9–10 mo after PNX.

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REFERENCES