Vascular growth and remodeling in compensatory lung growth following right lobectomy

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Le Cras, Timothy D., Lucas G. Fernandez, Patricia A. Pastura, and Victor E. Laubach. Vascular growth and remodeling in compensatory lung growth following right lobectomy. J Appl Physiol 98: 1140–1148, 2005.—Studies in animal models have shown that, following lobectomy (LBX), there is compensatory growth in the remaining lung. The vascular growth response following right LBX (R-LBX) is poorly understood. To test the hypothesis that arterial growth and remodeling occur in response to LBX, in proportion to the amount of right lung tissue removed, two (24% of lung mass; R-LBX2 group) or three right lobes (52% of lung mass; R-LBX3 group) were removed via thoracotomy from adult rats. Sham control animals underwent thoracotomy only. Arteriograms were generated 3 wk after surgery. The areas of the left lung arteriogram, arterial branching, length of arterial branches, arterial density, and arterial-to-alveolar ratios were measured. To determine whether R-LBX causes vascular remodeling and pulmonary hypertension, muscularization of arterioles and right ventricular hypertrophy were assessed. Lung weight and volume indexes were greater in R-LBX3 compared with sham but was 30% lower in R-LBX2. Arterial area, branch lengths, density, and arterial-to-alveolar ratios were measured in the left lung to determine whether pulmonary hypertension and vascular remodeling occur in proportion to the amount of lung tissue removed. Arterial growth was studied by angiography 3 wk after R-LBX. Arterial area, branch lengths, density, and arterial-to-alveolar ratios were measured in the left lung to assess arterial growth following compensatory lung growth. In addition, to determine whether pulmonary hypertension and vascular remodeling develop following removal of lung tissue, right ventricular (RV) hypertrophy and muscularization of arterioles were also assessed after removal of two or three right lung lobes.

MATERIALS AND METHODS

Animals and surgery. All animal procedures and protocols were approved by the Animal Care and Use Committee at the Cincinnati Children’s Hospital Research Foundation, Cincinnati, OH, and the Animal Care and Use Committee at the University of Virginia Health System, Charlottesville, VA. Adult male Sprague-Dawley rats (250–
300 g) were obtained from Charles River (Wilmington, MA). Body weights (BW), total lung weights, and individual lobe weights were measured in normal, unoperated rats to determine the percentage of lung mass that each lobe represented. R-LBX were performed on additional rats after a right thoracotomy, with the removal of two (upper and middle; R-LBX2) or three (upper, middle, lower; R-LBX3) right lung lobes. The infracardiac lobe was not removed in any group. Controls for this study were sham surgery rats in which a right thoracotomy was performed, but no lobes were removed. Surgery procedures and subsequent care of the rats were

![Image](image_url)

**Fig. 1.** Arteriograms following right lobectomy (R-LBX). A: arteriogram of left and right lungs of sham control animal (left). Arteriogram is shown of remaining right lobes (lower and infracardiac) and left lung after 2 right lobes were removed (R-LBX2; middle). Arteriogram after 3 right lobes were removed (R-LBX3; right) shows barium in the left lung, whereas no barium perfusion into the remaining right lobe (infracardiac) was observed. Arteriograms were performed 3 wk after surgery and are representative of 4–5 animals per group. The left lung is on the right side of the arteriograms. U, branch of right pulmonary artery supplying the upper right lobe; M, branch of right pulmonary artery supplying the middle right lobe; L, branch of right pulmonary artery supplying the lower right lobe; C, branch of right pulmonary artery supplying the infracardiac lobe; LPA, left pulmonary artery supplying left lung. B: arteriograms of left lungs from sham surgery controls and R-LBX groups after removal of R-LBX2 or R-LBX3. Arteriograms were performed 3 wk after surgery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Lung Weight Index, mg/g</th>
<th>Lung Volume Index, ml/kg</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Change</td>
<td>Initial</td>
</tr>
<tr>
<td>Sham controls</td>
<td>6</td>
<td>310±4</td>
<td>383±18</td>
<td>74±16</td>
</tr>
<tr>
<td>R-LBX2</td>
<td>5</td>
<td>310±3</td>
<td>392±11</td>
<td>82±11</td>
</tr>
<tr>
<td>R-LBX3</td>
<td>6</td>
<td>307±2</td>
<td>355±13</td>
<td>47±14†</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n, no. of animals. R-LBX2 and R-LBX3, right lobectomy with 2 or 3 lobes removed, respectively. †P < 0.05 vs. initial body weight.*
as previously described (14). BW were measured before surgery and at the time of death. Pulmonary vascular growth and remodeling were assessed 3 wk after surgery. Rats were killed using a pentobarbital sodium (26%) euthanasia solution (Fort Dodge Animal Health, Fort Dodge, IA).

Lung weight and volume indexes and hematocrit. Three weeks following surgery, tracheal inflation was performed on the study groups (sham, R-LBX2, and R-LBX3) with 4% paraformaldehyde at constant pressure (20 cmH2O). Lung volume was measured using the volume displacement method (27) and indexed to BW. Hematocrit

![Image](image-url)

A. Sham Control and R-LBX3

B. Arterial Area Index (Area / Body Weight)

C. Number of Visible Branches

D. Arterial Segment Length / Body Weight

E. Arterial Area Index vs. Lung Volume Index

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was measured in heparinized blood using a blood-gas analyzer (RapidLab, Bayer, EastWalpole, MA).

Arterial area and density, alveolar densities, and arterial-to-alveolar ratios. Arterial growth was assessed by performing barium arteriograms, as previously described (16). Briefly, a thoracotomy was rapidly performed, and heparin (10 units) was injected into the RV to prevent blood from clotting in the lungs. After tracheostomy, the lungs were gently inflated with air via a syringe, and a stainless steel gavage needle was inserted into the trachea. The lungs were inflated with the chest partially open, so that they just filled the thorax. Blood was flushed from the lungs with heparinized saline (1 U/ml) through a catheter inserted through the wall of the RV into the main pulmonary artery. A heated solution of gelatin and barium was infused into the main pulmonary arterial catheter at 74-mmHg pressure for at least 5 min. The main pulmonary artery was ligated under pressure with suture, and the lungs were inflation fixed by tracheal installation of 4% paraformaldehyde under constant pressure (25 cmH2O). After 48 h, the barium-filled arterial structure in the lungs was imaged by radiography, using a high-resolution X-ray machine (MX-20; Faxitron X-ray, Wheeling, IL) and high-resolution X-ray film (Microvision; AGFA, Greenville, SC). Radiographs were scanned and imaged using a flat-bed scanner with a transparency adapter. Quantitation of the arterial area (white area of arteriogram) was performed using Imagequant (Amersham Biosciences). The number of arterial branches visible on the arteriogram was counted for the first (apical) branch off the left pulmonary artery (LPA), and the length of arterial branches was measured using the measuring tool of Photoshop (Adobe), calibrated to a 1-cm line on the arteriogram. Arterial measurements (area and branch length) were indexed to BW to control for variations due to body size. The density of pulmonary arteries was assessed by counting barium-filled arteries (~30- to 120-μm external diameter) in five 4× fields of distal alveolar regions per animal (16). Alveoli were also counted in the same fields to determine alveolar density. The arterial and alveolar counts were indexed to the area of the field (2.4 mm²) to determine arterial density (arteries/mm²) and alveolar density (alveoli/mm²). The number of arteries per 100 alveoli was determined from the arterial and alveolar density data (24).

Arterial remodeling. To determine whether muscularization of small pulmonary arteries was increased in the remaining lung following PNX, immunohistochemical staining was performed for smooth-muscle α-actin on 5-μm sections of paraffin-embedded lung tissue fixed with 4% paraformaldehyde. A mouse monoclonal antibody (clone 1A4; Sigma) was used as previously described (16), and sections were lightly counterstained with hematoxylin before dehydration and mounting. Arterioles in alveolar ducts were identified (~30- to 80-μm external diameter) and then scored for muscularization by an observer blinded to the identity of the slides. Because the arteries contained barium, they could be distinguished from veins, as the barium preparation does not pass through the capillaries, and so the venous system does not contain barium (5). Arterioles in alveolar ducts were identified and then scored for muscularization (16). Arterioles were scored as either nonmuscular (NM; <50% surrounded by smooth muscle cells), partially muscular (PM; 50%–90% surrounded by smooth muscle cells but <100%), or fully muscular (FM; 100% surrounded by smooth muscle cells). Of the arterioles that were scored for muscularization, the percentage that were NM vs. PM vs. FM was compared with sham controls (4 animals per group; 30 arterioles per animal).

Pulmonary hypertension. RV hypertrophy was assessed as an index of pulmonary hypertension. Hearts were removed and dissected to isolate the free wall of the RV from the left ventricle and septum (LV+ S). The ratio of RV weight to BW and ratio of RV to LV+ S were used as an index of RV hypertrophy, which develops as a result of pulmonary hypertension.

Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed with a statistical software package (Statview, Abacus Concepts, Berkeley, CA). Statistical comparisons were made using ANOVA and post hoc tests (Fisher’s protected least significant difference test) or unpaired t-tests. P < 0.05 was considered significant.

RESULTS

Mortality and BW. Sham surgery was performed on 10 rats, and there were no deaths in this group (n = 10). R-LBX2 were removed from 10 rats; one died postsurgery before the time of euthanasia (final, n = 9). R-LBX3 were removed from 11 rats, with no deaths postsurgery (n = 11). Initial BW, BW at death, and change in BW over the 3-wk period following surgery were not different among R-LBX2 group, R-LBX3 group, and the sham surgery group (Table 1). BW increased in all three groups over the 3-wk period (Table 1).

Lobe weights, total lung weight, volume indexes, and hematocrit. To determine the contribution of lobe and lung weights to total lung tissue weight, the weights of individual lung lobes were measured in normal unoperated rats (n = 9). The total lung weight (left and right lungs) was 1.475 ± 0.163 g. The upper right lobe was 0.167 ± 0.007 g (11% of total lung mass), middle right lobe was 0.191 ± 0.006 g (13% of total lung mass), middle right lobe was 0.167 ± 0.006 g (13% of total lung mass), and left lung was 0.532 ± 0.019 g (36% of total lung mass). Therefore, removal of R-LBX2 constituted removal of ~24% of total lung mass, and removal of R-LBX3 constituted removal of ~52% of total lung mass.

Total lung weight and volume were measured in sham and LBX groups 3 wk after surgery (n = 5–6 animals per group). Lung weight and volume index increased 2.3- and 1.8-fold, respectively, in rats in which R-LBX3 were removed (Table 1), compared with sham controls (P < 0.05). Lung weight and volume index in rats that had R-LBX2 removed were increased; however, this did not reach statistical significance (Table 1; P > 0.05 vs. sham controls). Hematocrit measurements at death, 3 wk after surgery, were not different among the groups (Table 1).

Fig. 2. Analysis of arteriograms shows increased vascular area and length of arterial branches. A: example of arteriograms of left lungs from sham surgery control and following removal of R-LBX3. Vascular area was imaged by outlining the entire area of the left lung arteriogram (large box) and then performing area analysis of the scanned image. The number of branches was counted for the first branch (apical; small box) off the LPA (branches nos. 1–4). The length between the LPA and the first branch (segment A) and then first and second branch (segment B) was measured. B: histogram shows that the arterial area index [arterial area corrected to body weight (BW)] of the left lung increased in R-LBX2 and R-LBX3 groups compared with sham surgery controls. There was no significant difference between R-LBX2 and R-LBX3 groups. Data were derived from 4–5 animals in each group. *P < 0.05 vs. sham. C: histogram shows that the number of visible branches of the first arterial branch (apical) of the LPA on the arteriogram increased in rats where R-LBX3 were removed. Data were derived from 4–5 animals in each group. *P < 0.05 vs. sham. D: histogram shows that the length between the LPA and the first branch (segment A) of the first (apical) branch of the LV+ S was increased when R-LBX2 were removed (P > 0.05). The length between the first and second branches (segment B) increased after removal of 2 and 3 right lung lobes (*P < 0.05). Length of segment B was also higher in R-LBX3 vs. R-LBX2. Data were derived from 4–5 animals in each group. Arterial segment lengths were measured at ×3 magnification and indexed to BW. †P < 0.05 vs. R-LBX2. *P < 0.05 vs. sham. E: graph shows vascular area index plotted against lung volume index. *P < 0.05 vs. sham. Increases in vascular area correlate with increases in lung volume in R-LBX3 group. Values are means ± SE.
**Arteriograms.** Barium arteriograms were performed on four to five animals per group. A representative example of a whole lung (or remaining lung) arteriogram from each group is shown in Figure 1A. Radiography showed that, in the R-LBX3 group, where three lobes (upper, middle, and lower) were removed at the time of surgery, barium infusion into the infracardiac lobe was absent, and the lobe appeared atrophied when the lungs were removed at death. This indicates that blood flow to the remaining infracardiac lobe was compromised in the R-LBX3 animals. After whole lung arteriogram (or remaining lung) had been generated, the left lungs were dissected free and imaged (Fig. 1B).

**Arterial area, density, and arterial-to-alveolar ratios.** Total arterial growth was assessed by comparing the area of the left lung arteriogram indexed to BW, and Fig. 2A depicts an example of this. Total arterial area indexed to BW was increased in R-LBX2 (26 ± 6%) and R-LBX3 (47 ± 18%), compared with sham controls (Fig. 2B; P < 0.05). The number of arterial branches visible on the arteriogram was counted for the first branch (apical) off the LPA (Fig. 2A). The number of visible branches increased in R-LBX3 (P < 0.05), but a similar number of branches was seen in arteriograms of the left lungs of R-LBX2 animals compared with sham controls (Fig. 2C). The length between the LPA and the first branch (segment A) of the first (apical) branch off the LPA and between the first and second branches (segment B) were measured from the arteriograms and indexed to BW (Fig. 2A). The length of segment A increased 1.5-fold in R-LBX3 (P < 0.05), but not in R-LBX2 (P > 0.05), compared with sham controls (Fig. 2D). The length of segment B increased 1.3- and 1.7-fold in R-LBX2 and R-LBX3, respectively, compared with sham controls (P < 0.05; Fig. 2D). Length of segment B was also higher in R-LBX3 vs. R-LBX2 (P < 0.05; Fig. 2D).

Vascular area index was plotted against lung volume index and shows that increases in vascular area correlate with increases in lung volume in R-LBX3 group (Fig. 2E). The number of arteries and alveoli was counted in distal (alveolar) regions of barium-perfused lungs (Fig. 3 and Table 2). The number of arteries per millimeter squared was not significantly different between R-LBX2 and sham controls (P > 0.05), but was 53% lower in R-LBX3 (P < 0.05). The number of alveoli per millimeter squared was not different between R-LBX2 and sham controls (P > 0.05), but was 33% lower in R-LBX3 (P < 0.05). The number of arteries per 100 alveoli was not different between R-LBX2 and sham controls (P > 0.05), but was 30% lower in R-LBX3 (P < 0.05) (Table 2).

**Pulmonary hypertension.** Consistent with increased muscularization of small pulmonary arteries in R-LBX3, there was additional evidence of pulmonary hypertension in the R-LBX3 group as the RV-to-BW ratio was increased 1.5-fold, compared with sham controls and R-LBX2 (Fig. 5; P < 0.05). The RV to LV+S weight was increased 1.7-fold in R-LBX3 (0.472 ± 0.03) relative to sham and LBX2 groups (0.276 ± 0.01 and 0.305 ± 0.01, respectively; P < 0.05). There was no evidence of RV hypertrophy in R-LBX2, consistent with muscularization of small pulmonary arteries in R-LBX2 being similar to sham, as shown in Fig. 4B.

**DISCUSSION**

In the present study, removal of three right lobes (R-LBX3 group) compromised blood flow to the remaining right lobe, because barium infusion into the infracardiac lobe was not observed, and the lobe either was absent or appeared atrophied. As a result, the R-LBX3 group in this study should be considered, at least in terms of pulmonary blood flow, to have compromised blood flow to all of the right lobes and, therefore, to represent a total right PNX (63% of lung mass). Lung weight and volume increased approximately twofold in the R-LBX3...
group, which agrees with previous studies in dogs in which total right PNX was performed (12, 29). In our study, angiography showed that arterial growth occurred in the left lung following R-LBX. Arterial area increased 26% when two right lobes were removed, and 47% when three lobes were removed. These findings correlate well with the study by Hsia et al. (12) in which right PNX in dogs caused a 55% increase in endothelium and 34% increase in capillary surface area. Arterial density and arterial-to-alveolar ratios were similar in the left lungs of the R-LBX2 group and sham controls, but were lower in R-LBX3 animals. Hence, although vascular growth occurred in the R-LBX3 group, the ratio of arteries to alveoli was lower than that of the sham controls and R-LBX2 animals.

Angiography in the R-LBX2 group showed that vascular flow to the remaining right lung lobes (lower and infracardiac) was not compromised and that there was a small increase in arterial area in the left lung. Whereas there was not an increase in the length of the more proximal arterial branches (segment

Table 2. Arterial density, alveolar density, and arterial-to-alveolar ratios in left lung

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Arterial Density, arteries/mm²</th>
<th>Alveolar Density, alveoli/mm²</th>
<th>Arteries per 100 Alveoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham controls</td>
<td>4</td>
<td>7.18 ± 0.46</td>
<td>221 ± 19</td>
<td>3.3 ± 0.15</td>
</tr>
<tr>
<td>R-LBX2</td>
<td>4</td>
<td>5.63 ± 0.63</td>
<td>198 ± 12</td>
<td>2.8 ± 0.26</td>
</tr>
<tr>
<td>R-LBX3</td>
<td>5</td>
<td>3.34 ± 0.15*</td>
<td>147 ± 9*</td>
<td>2.3 ± 0.23*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. *P < 0.05 vs. sham controls.

Fig. 4. Vascular remodeling and muscularization following R-LBX. A: immunostaining for smooth muscle α-actin of left lung sections from sham control and R-LBX3. Immunostaining for smooth muscle α-actin was assessed in rats 3 wk after sham surgery or after removal of R-LBX2 (data not shown) or R-LBX3. Immunostaining for smooth muscle α-actin detected smooth muscle cells associated with vessels (arrows), as well as alveolar myofibroblasts. Open arrows indicate small pulmonary arteries associated with alveolar ducts with <50% muscularization (nonmuscular). Solid arrows indicate fully muscularized arterioles associated with alveolar ducts. Arteries were distinguished from veins as they contained barium (solid material filling lumen), whereas veins did not. R-LBX2 immunostaining (not shown) appears similar to sham. Bar = 100 μm. B: histogram of muscularization of small pulmonary arteries after immunostaining for smooth muscle α-actin. Morphometric analysis was performed after immunostaining for smooth muscle α-actin on lung sections from rats 3 wk after sham surgery or removal of R-LBX2 or R-LBX3. Analysis was performed on 30- to 80-μm-diameter arteries associated with alveolar ducts. Arteries were scored as either nonmuscular (NM; <50% of perimeter muscularized), partially muscular (PM; >50% but <100% muscularized), or fully muscularized (FM; 100% of perimeter muscularized). Data were derived from 4–5 animals in each group. Values are means ± SE. *P < 0.05 vs. sham surgery control.
A) in the R-LBX2 group, the length of the next generation of branches (segment B) did increase. Arterial and alveolar densities and arterial-to-alveolar ratios in the R-LBX2 group were similar to those in sham, suggesting that, whereas proximal growth in these animals was limited, significant distal arterial growth occurred. However, lung weight and volume indexes were not significantly increased in the R-LBX2 group, which raises the possibility that the lung can make adjustments in the vasculature without gross changes in lung weight or volume. In the study by Hsia et al. (12), right PNX in dogs caused a 72% increase in left lung volume, 55% increase in endothelium, 43% increase in capillary blood volume, and 34% increase in capillary surface area. Our findings in rats correlate well with those of Hsia et al. in dogs, as removal of three right lobes resulted in an ~80% increase in lung volume and 47% increase in arterial area and growth of proximal and distal arterial branches. In addition, more arterial branches were visible on the arteriograms in the R-LBX3 group. Because branches of this size are usually formed prenatally, this likely represents an increase in size (diameter) of these vessels (rather than development of new branches) such that they are now visible on the arteriogram as they accommodate more of the barium used for imaging. However, arterial density and arterial-to-alveolar ratios in the distal lung of R-LBX3 animals were reduced. This indicates that, while arterial growth occurred, it did not generate the normal ratio of arteries to alveoli in the distal lung. In addition, alveolar density was also reduced in the R-LBX3 group, indicating an increase in alveolar size. Increased alveolar size following PNX has been reported in a number of previous studies, particularly in older animals (2, 10, 11, 12). Hislop et al. (10) transplanted right cardiac lung lobes from adult rats into the left hemithorax of juvenile rats after left PNX. Six months after transplantation, both the recipient right lung and the transplanted right lung were larger than normal. They reported that this was due to an increase in alveolar number in the recipient right lung and to an increase in the size of alveoli in the transplanted cardiac lobe (10).

In preliminary studies, we have also examined the arterial response of the right lobes to left PNX (8). Three weeks after surgery, angiography of the right lung lobes showed that all lobes had increases in arterial area compared with right lung lobes from sham controls. The upper and middle right lobes also had a higher arterial area than the lower and infracardiac lobes, showing that there was a differential response, in that the upper lobes displayed greater arterial growth than the lower lobes (8). This correlated with higher levels of proliferating nuclear cell antigen in these lobes (7). In contrast, arterial growth did not correlate well with lung weight and volume indexes (8).

In the present study, muscularization of pulmonary arterioles increased, and there was RV hypertrophy following removal of three lobes (R-LBX3 group), which indicates that pulmonary hypertension developed. This finding is consistent with a previous study by Takeda et al. (28) in which hemodynamic responses in dogs following right PNX were reported. In immature foxhounds which underwent right PNX, measurements of cardiopulmonary function during treadmill exercise at maturity (1 yr of age) showed that, while maximal oxygen uptake, cardiac output, arterial and mixed-venous blood gases, and arteriovenous oxygen extraction were normal during exercise, mean pulmonary arterial pressure and resistance were elevated at a given cardiac output. Also reported in this study was a comparison of mean pulmonary arterial pressure between dogs pneumonectomized as adults with pulmonary arterial pressures in dogs pneumonectomized as puppies (28). In dogs pneumonectomized as adults, pulmonary arterial pressure at peak exercise was ~60% higher than that in sham controls, and maximal cardiac output was ~25% lower, whereas in dogs pneumonectomized as puppies, pulmonary arterial pressure was ~20% higher at peak exercise, and maximal cardiac output was not reduced. Both the present study and the study by Takeda et al. (28) suggest that loss of three to four right lobes leads to vascular remodeling and pulmonary hypertension. Loss of two right lobes in our study did not cause vascular remodeling or pulmonary hypertension. Our findings suggest that pulmonary vascular resistance was increased following R-LBX3 and that this was significant enough to cause pulmonary hypertension, despite compensatory growth. Factors potentially contributing to increased pulmonary vascular resistance in this study include the reduction in distal pulmonary arterial density and increased muscularization of small pulmonary arteries. Increased shear stress, due to reductions in vascular surface area following PNX, such as in the present study, has been shown to induce vascular remodeling in other models (6). In disease states such as emphysema in which alveolar and vascular area is lost, vascular remodeling and pulmonary hypertension contribute to morbidity and mortality.

A limitation of our study was that the angiography technique that was used is a two-dimensional imaging technique, whereas the pulmonary arterial system is a three-dimensional structure. In the future, three-dimensional imaging techniques, such as computer axial tomography scan and MRI, may be possible once adapted to small animals such as rats and mice. However, currently, most computer axial tomography scan and MRI equipment lack the ability to give fine enough detail to permit analysis of the vasculature of these animals. In this study, we used the first (apical) branch off of the LPA to be representative of the vascular growth that was apparent in all other areas of the lung. The length of branches was easily measured for
this artery, as it did not overlap with other arteries on the radiograph. Another potential limitation of this study is that recruitment of existing vessels that are not normally perfused under basal conditions could have contributed to the increase in vascular area that was observed in the R-LBX rats. However, the barium mixture was infused at high pressure (74 mmHg), which should have perfused these vessels, and all arteries were filled with barium upon histological examination during the course of the arterial density counts. In addition, we performed immunostaining for von Willibrand factor, which stains the endothelium of vessels, and then repeated the vessel density counts. With this technique, arterial counts were similar to counts obtained by counting barium-filled vessels (data not shown).

The molecular and cellular mechanisms driving the vascular response were not assessed in our study. Previous studies have shown that loss of endothelial nitric oxide synthase and treatment with a nitric oxide synthase inhibitor prevents compensatory lung growth following left PNX in mice (17). Nitric oxide is an essential mediator of vascular endothelial growth factor-mediated angiogenesis (22). Retinoids have been shown to enhance lung growth after PNX (14) and to induce the formation of alveoli in rats with elastase-induced emphysema and steroid-induced inhibition of septation (18–20). In a recent study, Yan et al. (31) treated adult dogs with retinoic acid following right PNX and reported that endothelial cells and capillary volume increased, but that lung volume and epithelial interstitial volumes did not. Interestingly, Yan et al. reported the appearance of double septal capillary profiles, which are typical of the developing lung, but not normally observed in the adult lung. Yan et al. suggested that retinoid acid treatment may cause the alveolar capillaries to revert to an immature state. In the present study, arterial growth following LBX may be a primary response or a secondary response to changes in lung volume and alveolar size and number. In the neonatal lung, inhibition of angiogenesis disrupted postnatal alveolarization, indicating that vascular development is necessary for alveologenesis (13).

Physiological factors, which regulate and are responsible for the stimulus for compensatory lung growth, are poorly understood. Several potential stimuli have been proposed to regulate the onset and progression of compensatory growth (9, 12), including the following: 1) postoperative changes in tissue inflation and mechanical strain; 2) elevated blood flow in the remaining vasculature; 3) hypoxemia; and 4) release of endocrine, paracrine, and/or autocrine factors. A study by McBride and coworkers (21) indicates that elevated blood flow in the remaining lung following PNX is not the primary regulator of compensatory lung growth. McBride and coworkers placed a band around the segment of the pulmonary artery leading to the caudal lobe of the left lung in ferrets. The band was calibrated so that, following right PNX, increased blood flow to that lobe was prevented, whereas the cranial lobe accommodated the remainder of the cardiac output. Compensatory lung growth was observed in all lobes, including the lobe in which blood flow did not increase after PNX, suggesting that elevated blood flow is not the primary stimulus for compensatory lung growth.

In summary, R-LBX induced a proportionate arterial growth response in the left lung of adult rats, including increased length of arteries. Although arterial growth was observed in the left lung following removal of three right lobes, it did not generate the normal ratio of distal arteries to alveoli. Removal of three right lobes also caused vascular remodeling and pulmonary hypertension, whereas removal of two right lobes did not. Our results indicate that different arterial responses occur, depending on the amount of lung tissue removed.

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