VASCULAR GROWTH AND REMODELING
IN COMPENSATORY LUNG GROWTH
FOLLOWING RIGHT LOBECTOMY

Timothy D. Le Cras\textsuperscript{1}
Lucas G. Fernandez\textsuperscript{2}
Patricia A. Pastura\textsuperscript{1}
Victor E. Laubach\textsuperscript{2}

\textsuperscript{1}Division of Pulmonary Biology,
Cincinnati Children’s Hospital Medical Center & Department of Pediatrics,
University of Cincinnati, Cincinnati, Ohio

and

\textsuperscript{2}Department of Surgery, University of Virginia Health System,
Charlottesville, Virginia.

Correspondence to:
Tim Le Cras, Division of Pulmonary Biology,
Cincinnati Children’s Hospital Medical Center,
3333 Burnet Ave., Cincinnati, Ohio 45229-3039
Tel # 513-636-8151 Fax # 513-636-7868
Email: tim.lecras@cchmc.org

Running title: Vascular Growth and Remodeling Following Right
Pneumonectomy
ABSTRACT

Studies in animal models have shown that following lobectomy, there is compensatory growth in the remaining lung. The vascular growth response following right lobectomy (R-LBX) is poorly understood. To test the hypothesis that arterial growth and remodeling occurs 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 three weeks after surgery. The area of the left lung arteriogram, arterial branching, length of arterial branches, arterial density, and arterial to alveolar ratios were measured. To determine if R-LBX causes vascular remodeling and pulmonary hypertension, muscularization of arterioles and right ventricular hypertrophy were assessed. Lung weight and volume indices were greater in R-LBX3. Arterial area of the left lung increased 26% in R-LBX2 and 46% in R-LBX3. The length of large arteries increased in R-LBX3, and to a lesser extent in R-LBX2. The ratio of distal pulmonary arteries to alveoli was similar after R-LBX2 compared to sham, but was 30% lower in R-LBX3. Muscularization of arterioles increased after R-LBX3, but not in R-LBX2. Right ventricular hypertrophy increased 50-70% in R-LBX3, but not in R-LBX2. While removal of three right lung lobes induced arterial growth in the left lungs of adult rats, which was proportionate to the number of lobes removed, the ratio of distal pulmonary arteries to alveoli was not normal, and vascular remodeling and pulmonary hypertension developed.

Keywords: pulmonary hypertension; lung resection; vascular remodeling; emphysema
INTRODUCTION:

Compensatory lung growth following pneumonectomy (PNX) has been reported in a variety of species. Variations in the response are seen depending on the species, age, sex and also hormonal status of the animal (11,29,3,25,26,10,12,30). Left PNX in rats increases the growth of the right lung 8-fold above control, with normal lung mass achieved two weeks after surgery (26). Removal of right lung lobes (right lobectomy: R-LBX) produces a similar response in the left lung (29,1,4,28), with removal of the entire right lung resulting in almost a doubling of left lung mass and volume (28,12). Compensatory lung growth has also been reported in humans, particularly in children who have undergone PNX or LBX (23,15).

In small mammals, particularly rats and mice, studies have shown that generally there is complete restoration of normal total lung mass following PNX (26,4,17,29). Furthermore, growth of the distal alveolar region of the remaining lung following PNX can be rapid and complete, leading to a restoration of total lung volume, compliance, mass, alveolar number, and normal lung cell populations (26). However, the response of the pulmonary vasculature in compensatory lung growth is not well characterized. In the neonatal rat lung, angiogenesis has been shown to be vital for normal postnatal alveologenesis to occur (13). Hsia et al. showed that endothelium and capillary blood volume and surface area increased following right pneumonectomy in dogs (12). Takeda et al. have reported elevated mean pulmonary arterial pressure and resistance during exercise in immature foxhounds which underwent right PNX (28). However, whether arterial remodeling occurs after R-LBX and the arterial growth response is still poorly understood.

The goal of this study was to characterize the arterial response in the left lung following R-LBX in adult rats and to determine; 1) the extent of the arterial growth response, and 2) whether lobectomy causes vascular remodeling and pulmonary hypertension. To address these questions two or three right lung lobes were removed to test the hypothesis that arterial growth and remodeling occurs in proportion to the amount of lung tissue removed. Arterial growth was studied by angiography three weeks 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 if pulmonary hypertension and vascular remodeling develop following removal of lung tissue, right ventricular hypertrophy and muscularization of arterioles were also assessed after removal of two or three right lung lobes.
METHODS & MATERIALS:

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, Ohio and the Animal Care and Use Committee at the University of Virginia Health System, Charlottesville, Virginia. Adult male Sprague Dawley rats (250-300g) were obtained from Charles River (Wilmington, MA). Body weights, total lung weights and individual lobe weights were measured in normal un-operated rats to determine the percentage of lung mass which each lobe represented. Right lobectomies (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 infra-cardiac 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 as previously described (14). Body weights were measured prior to surgery and at the time of sacrifice. Pulmonary vascular growth and remodeling was assessed three weeks after surgery. Rats were sacrificed using a sodium pentobarbital (26%) euthanasia solution (Fort Dodge Animal Health, Fort Dodge, Iowa).

Lung weight and volume indices 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 (20cm H₂O). Lung volume was measured using the volume displacement method (27) and indexed to body weight. Hematocrit was measured in heparinized blood using a blood gas analyzer (“RapidLab”, Bayer Corp., 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 (10U) was injected into the right ventricle 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 (1U/ml) through a catheter inserted through the wall of the right ventricle into the main pulmonary artery. A heated solution of gelatin and barium was infused into the main pulmonary artery catheter at 74mm Hg pressure for at least 5 minutes. 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 (20cm H₂O). After 48 hours, the barium-filled arterial structure in the lungs was imaged by radiography, using a high resolution X-ray machine (MX-20; Faxitron X-ray Corp., Wheeling, IL) and high resolution X-ray film (Microvision; AGFA Corp., 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, visible branch number, and branch length) were indexed to body weight to control for variations due to body size. The density of pulmonary arteries was assessed by counting barium-filled arteries (~30-120µm external diameter) in 5 high powered 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 (2.4mm²) to determine arterial density (arteries per mm²) and alveolar density (alveoli per 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 5µm 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 counter-stained with hematoxylin before dehydration and mounting. Arterioles in alveolar ducts were identified (~30-80µm external diameter) and then scored for muscularization by an
observer blinded to the identity of the slides. Since 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 non-muscular (NM; <50% surrounded by smooth muscle cells), partially muscular (PM; >50% 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 versus PM versus FM was calculated for each animal (4 animals per group; 30 arterioles per animal).

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

**Statistical analysis:** Data are presented as mean±SEM. Statistical analysis was performed with a statistical software package (Statview, Abacus Concepts, Berkely, CA). Statistical comparisons were made using analysis of variance (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 body weights: Sham surgery was performed on 10 rats, and there were no deaths in this group (n=10). Two right lung lobes (upper and middle lobes; R-LBX2) were removed from 10 rats, one died post-surgery before the time of sacrifice (final n=9). Three right lung lobes (upper, middle and lower; R-LBX3) were removed from 11 rats, with no deaths post-surgery (n=11). Initial body weight (BW), BW at sacrifice, and change in BW over the three week period following surgery were not different between R-LBX2 group, R-LBX3 group and the sham surgery group (Table 1). Body weights increased in all three groups over the three week period (Table 1).

Lobe weights, total lung weight and volume indices 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 un-operated rats (n=9). The total lung weight (left and right lungs) was 1.475±0.163g. The upper right lobe was 0.167±0.007g (11% of total lung mass); middle right lobe 0.191±0.006g (13% of total lung mass); lower right lobe 0.417±0.018g (28% of total lung mass), infra-cardiac right lobe 0.167±0.019 (11% of total lung mass), left lung 0.532±0.019 (36% of total lung mass). Therefore, removal of the upper two right lobes (R-LBX2) constituted removal of approximately 24% of total lung mass, and removal of the three upper right lobes (R-LBX3) constituted removal of approximately 52% of total lung mass.

Total lung weight and volume were measured in sham and lobectomy groups three weeks after surgery (n=5-6 animals per group). Lung weight and volume index increased 2.3-fold and 1.8-fold, respectively, in rats in which 3 right lobes were removed (R-LBX3) (Table 1), compared to sham controls (P<0.05). Lung weight and volume index in rats which had 2 right lobes removed (R-LBX2) were increased, however this did not reach statistical significance (Table 1; P>0.05 versus sham controls). Hematocrit measurements at sacrifice, 3 weeks after surgery, were not different among the groups (Table 1).

Arteriograms: Barium arteriograms were performed on 4-5 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 infra-cardiac lobe was absent, and the lobe appeared atrophied when the lungs were removed at sacrifice. This indicates that blood flow to the remaining infra-cardiac 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 (Figure 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 body weight, and Figure 2a depicts an example of this. Total arterial area indexed to body weight was increased in R-LBX2 (26±6%) and R-LBX3 (47±18%), compared to sham controls (Figure 2b; P<0.05). The number of arterial branches visible on the arteriogram was counted for the first branch (apical) off the LPA (Figure 2a). The number of visible branches increased in R-LBX3 (P<0.05), but a similar number of branches were seen in arteriograms of the left lungs of R-LBX2 animals compared to sham controls (Figure 2c). The length between the LPA and the first branch (segment A) of the first (apical) branch of the LPA, and the first and second branch (segment B) was measured from the arteriograms and indexed to body weight (Figure 2a). The length of segment A increased 1.5-fold in R-LBX3 (P<0.05), but not in R-LBX-2 (P>0.05) compared to sham controls (Figure 2d). The length of segment B increased 1.3- and 1.7-fold in R-LBX2 and R-LBX3, respectively, compared to sham controls (P<0.05; Figure 2d). Length of segment B was also higher in R-LBX3 versus R-LBX2 (P<0.05; Figure 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 (Figure 2e). The number of arteries and alveoli were counted in distal (alveolar) regions of barium-perfused lungs (Figure 3 and Table 2). The number of arteries per $mm^2$ was lower but 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 $mm^2$ 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).
Arterial remodeling: Immunostaining for smooth muscle α-actin was performed on sections from barium-perfused lungs (Figure 4a). Morphometric analysis showed that muscularization of arterioles associated with alveolar ducts increased in R-LBX3 (P<0.05), but not in R-LBX2 (P>0.05), compared to sham controls (Figure 4b). The percentage of fully muscularized arterioles significantly increased in R-LBX3 compared to sham (P<0.05), while the percentage of non-muscular arterioles was significantly decreased in R-LBX3 (P<0.05)(Figure 4b).

Pulmonary hypertension: Consistent with increased muscularization of small pulmonary arteries in R-LBX3, there was additional evidence of pulmonary hypertension in the R-LBX3 groups as the RV/BW ratio was increased 1.5-fold, compared to sham controls and R-LBX2 (Figure 5; P<0.05). The RV to LV+S weight was increased 1.7-fold in R-LBX-3 (0.472±0.03) relative to sham and LBX-2 groups (0.276±0.01 and 0.305±0.01, respectively; P<0.05). There was no evidence of right ventricular hypertrophy in R-LBX2, consistent with muscularization of small pulmonary arteries in R-LBX2 being similar to sham, as shown in Figure 4b.
DISCUSSION:

In the present study, removal of three right lobes (R-LBX3 group) compromised blood flow to the remaining right lobe, since barium infusion into the infra-cardiac lobe was not observed and the lobe was either 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 the right lobes and therefore to represent a total right pneumonectomy (63% of lung mass). Lung weight and volume increased approximately two-fold in the R-LBX3 group, which agrees with previous studies in dogs in which total right PNX was performed (12,29). In our study angioography showed that arterial growth occurred in the left lung following right lobectomy. Arterial area increased 24% when two right lobes were removed, and 46% 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 infra-cardiac) was not compromised, and that there was a small increase in arterial area in the left lung. While there was not an increase in the length of the more proximal arterial branches (segment A) in the R-LBX2 group, the length of the next generation of branches (segment B) did increase. Arterial and alveolar densities and artery to alveoli ratios in the R-LBX2 group were similar to sham, suggesting that while proximal growth in these animals was limited, significant distal arterial growth occurred. However, lung weight and volume indices 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 (12), as removal of three right lobes resulted in an ~80% increase in lung volume and 46% 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. Since 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 pneumonectomy 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 pneumonectomy. 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 (7). Three weeks after surgery, angiography of the right lung lobes showed that all lobes had increases in arterial area compared to right lung lobes from sham controls. The upper and middle right lobes also had a higher arterial area than the lower and infra-cardiac lobes, showing that there was a differential response, in that the upper lobes displayed greater arterial growth than the lower lobes (7). This correlated with higher levels of proliferating nuclear cell antigen (PCNA) in these lobes (8). In contrast arterial growth did not correlate well with lung weight and volume indices (7).

In the present study muscularization of pulmonary arterioles increased and there was right ventricular hypertrophy following removal of three lobes (R-LBX3 group), which indicates that pulmonary hypertension developed. This finding is consistent with that of 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 year 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 artery pressure between dogs pneumonectomized as adults, with pulmonary artery pressures in dogs pneumonectomized as puppies (28). In dogs pneumonectomized as adults, pulmonary artery pressure at peak exercise was ~60% higher than sham controls and maximal cardiac output was ~25% lower, whereas in dogs pneumonectomized as puppies, pulmonary artery 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 artery 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 which 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 (CAT scan) and magnetic resonance imaging (MRI), may be possible once adapted to small animals such as rats and mice. However, currently most CAT 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 the LPA, to be representative of the vascular growth that was apparent in all other areas of the lung. The length of branches were 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 (74mmHg), which should have perfused these vessels, and all arteries were filled with barium upon histologic 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 vessels density counts. Using 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 (eNOS) 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 and interstitial volumes did not. Interestingly, Yan et al. (31) report 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 (31) suggest that retinoic 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 alveogenesis (13).
Physiologic 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; 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 co-workers indicates that elevated blood flow in the remaining lung following PNX is not the primary regulator of compensatory lung growth (21). McBride and coworkers placed a band around the segment of the pulmonary artery leading to the caudal lobe of the left lung in ferrets (21). The band was calibrated so that following right PNX increased blood flow to that lobe was prevented, while 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. While 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.
ACKNOWLEDGEMENTS:

This work was supported by NIH HL72894 (TDLC), American Lung Association Career Investigator Award CI-31-N (TDLC), NIH 5R01 HL67780 (VEL) and a Virginia Thoracic Society award CI-52-N (VEL).
REFERENCES:


FIGURE LEGENDS:

Figure 1: Arteriograms following right lobectomy.

Panel a: Arteriogram of left and right lungs of sham control animal (left panel). Arteriogram of remaining right lobes (lower and infra-cardiac) and left lung after 2 right lobes were removed (Right LBX 2; middle panel). Arteriogram after 3 right lobes were removed (Right LBX 3; right panel) showing barium in the left lung, whereas no barium perfusion into the remaining right lobe (infra-cardiac) was observed. Arteriograms were performed 3 weeks 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 infra-cardiac lobe; LPA = left pulmonary artery supplying left lung.

Panel b: Arteriograms of left lungs from sham surgery controls and right lobectomy groups after removal of two (R-LBX2) or three (R-LBX3) right lobes. Arteriograms were performed 3 weeks after surgery.

Figure 2: Analysis of arteriograms shows increased vascular area and length of arterial branches.

Panel a. An example of arteriograms of left lungs from sham surgery control and following removal of 3 right lobes (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 left pulmonary artery (LPA)(branches numbers 1-4). The length between the LPA and the first branch (segment A) and then first and second branch (segment B) (arrows) was measured.

Panel b. Histogram shows that arterial area index (arterial area corrected to body weight) of the left lung increased in R-LBX2 and R-LBX3 groups compared to 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 versus sham.
Panel c. Histogram shows that the number of visible branches of the first arterial branch (apical) of the left pulmonary artery on the arteriogram, increased in rats where 3 right lung lobes (R-LBX3) were removed. Data were derived from 4-5 animals in each group. *P<0.05 versus sham.

Panel d. Histogram shows that the length between the left pulmonary artery (LPA) and the first branch (Segment A) of the first (apical) branch of the LPA increased in rats when 3 right lung lobes (R-LBX3) were removed (P<0.05), but not when 2 right lung lobes (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 versus R-LBX2. Data were derived from 4-5 animals in each group. †P<0.05 versus R-LBX2. *P<0.05 versus sham.

Panel e. Graph shows vascular area index plotted against lung volume index. *P<0.05 versus sham. Increases in vascular area correlate with increases in lung volume in R-LBX3 group.

Figure 3: Lung histology following right lobectomy.
Lung histology shows barium-filled arteries (*) in the distal lung of sham and R-LBX3, while veins do not fill with barium (v). Lung sections were stained with hematoxylin and eosin after barium-perfusion and fixation. R-LBX2 histology (not shown) appears similar to sham. Bar =100 µm.

Figure 4: Vascular remodeling and muscularization following right lobectomy.
Panel 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 weeks after sham surgery, or after removal of two (R-LBX2) (data not shown) or three right lung lobes (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 (non-muscular). Solid arrows indicate fully muscularized arterioles associated with alveolar ducts. Arteries were distinguished from veins as they contained
barium (solid material filling lumen), while veins did not. R-LBX2 immunostaining (not shown) appears similar to sham. Bar = 100 μm.

**Panel 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 weeks after sham surgery, or removal of two (R-LBX2) or three right lung lobes (R-LBX3). Analysis was performed on 30-80 μm diameter arteries associated with alveolar ducts. Arteries were scored as either non-muscular (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. *P <0.05 versus sham surgery control.

**Figure 5: Pulmonary hypertension following right lobectomy.** Right ventricular hypertrophy was assessed in rats 3 weeks after sham surgery or removal of two (R-LBX2) or three right lung lobes (R-LBX3). Right ventricle weight (RV) was measured and the ratio of this weight to body weight (RV/BW) represents an index of pulmonary hypertension. Data were derived from 5-10 animals in each group. *P <0.05 versus sham and 2 R-LBX.
Sham Control

R-LBX2

R-LBX3

Fig#1a.
Sham Control  R-LBX3

1st apical branch off LPA
- visible branches (1, 2, 3, 4)
- branch segment lengths (arrows; A and B)

vascular area (white)
Arterial Area Index
(Area / Body Weight)

Sham R-LBX2 R-LBX3

Fig#2b Le Cras et al

by 10.220.33.4 on October 14, 2017 http://jap.physiology.org/ Downloaded from
Le Cras et al

**Number of Visible Branches**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>R-LBX2</th>
<th>R-LBX3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Arterial Segment Length / Body Weight (mm/g×3)

Segment A (LPA to 1st branch)
Segment B (1st-2nd branch)

Sham
R-LBX2
R-LBX3
Le Cras et al

Fig #2e

Arterial Area Index vs. Lung Volume Index

- R-LBX3
- R-LBX2
- Sham

* statistically significant difference
Sham Control  

R-LBX3

Le Cras et al
Le Cras et al

Sham Control

R-LBX3

Fig#4a
Percentage of Small Pulmonary Arteries (%)
Le Cras et al

RV / BW

Sham

R-LBX2

R-LBX3

*
Table 1: Body weight, lung weight, lung volume, and hematocrit.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</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></td>
<td>Initial Final</td>
<td>Change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham Controls</td>
<td>6</td>
<td>310±4 383±18</td>
<td>74±16#</td>
<td>2.55±0.20</td>
<td>16.2±0.5</td>
</tr>
<tr>
<td>R-LBX2</td>
<td>5</td>
<td>310±3 392±11</td>
<td>82±11#</td>
<td>3.28±0.33</td>
<td>18.4±0.8</td>
</tr>
<tr>
<td>R-LBX3</td>
<td>6</td>
<td>307±2 355±13</td>
<td>47±14#</td>
<td>5.88±0.62*</td>
<td>29.3±1.3*</td>
</tr>
</tbody>
</table>

* P<0.05 versus sham controls
# P<0.05 versus initial body weight
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/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>

*P<0.05 versus sham controls