Major Technical Advances

Longitudinal, Noninvasive Monitoring of Compensatory Lung Growth in Mice after Pneumonectomy via ^3He and ^1H Magnetic Resonance Imaging

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In rodents and some other mammals, partial pneumonectomy (PNX) of adult lungs results in rapid compensatory lung growth. In the past, quantification of compensatory lung growth and realveolarization could only be accomplished after killing the animal, removal of lungs, and histologic analysis of lungs at single time points. Hyperpolarized ^3He diffusion magnetic resonance imaging (MRI) allows *in vivo* morphometry of human lungs; our group has adapted this technique for application to mouse lungs. Through imaging, we can obtain maps of lung microstructural parameters that allow quantification of morphometric and physiologic measurements. In this study, we employed our ^3He MRI technique to image *in vivo* morphometry at baseline and to serially assess compensatory growth after left PNX of mice. ^1H and hyperpolarized ^3He diffusion MRI were performed at baseline (pre-PNX), 3-days, and 30-days after PNX. Compared with the individual mouse's own baseline, MRI was able to detect and serially quantify changes in lung volume, alveolar surface area, alveolar number, and regional changes in alveolar size that occurred during the course of post-PNX lung growth. These results are consistent with morphometry measurements reported in the literature for mouse post-PNX compensatory lung growth. In addition, we were also able to serially assess and quantify changes in the physiologic parameter of lung compliance during the course of compensatory lung growth; this was consistent with flexiVent data. With these techniques, we now have a noninvasive, *in vivo* method to serially assess the effectiveness of therapeutic interventions on post-PNX lung growth in the same mouse.

Keywords: helium; magnetic resonance imaging; pneumonectomy; neoalveolarization

Pneumonectomy (PNX), the surgical removal of all or major parts of a lung, is an established model of compensatory lung growth (1–3). The lung mass, structure, and function are restored by compensatory lung growth induced by PNX; the

CLINICAL RELEVANCE

This work uses a new imaging technique to visualize and quantitate neoalveolarization in the mouse after pneumonectomy. This opens avenues of inquiry related to lung injury and repair, normal and compensatory lung growth, and alveolar simplification during development.

PNX model is widely used to characterize the sources, mechanisms, and functional limits of compensatory lung growth. It is believed that mechanical stress and strain, in combination with a variety of growth factors and hormones, are key signals to induction of post-PNX compensatory lung growth (3). Understanding the time course and the mechanism of compensatory lung growth will shed light on understanding the pathophysiology of lung regeneration and growth. It may also provide insight into the development of novel therapeutic strategies for lung regeneration in various human lung diseases.

In humans, lung regeneration after PNX rarely occurs in adults, but it has been observed in children and, to a lesser degree, in adolescents (4–6). In animals, compensatory lung growth has been intensively studied in different species, including dogs, rabbits, ferrets, rats, and mice (7–12). In adult dogs, significant but incomplete compensatory growth occurs after major lung resection (> 50%) (7, 13). Compared with large mammals, rodents have more rapid response to PNX, and the time course of the normalization is much shorter, because somatic growth continues throughout their lifespan. This makes them of particular interest to the pulmonary community. Although intensive research has been performed on rats (14–18), the detailed characterization of post-PNX lung growth by histology was not reported in mice until recently. Fehrenbach and colleagues (19) have demonstrated that mice undergoing unilateral PNX have the capacity for a rapid and complete compensatory lung growth, which includes cellular proliferation and full normalization of lung volume, alveolar surface area, and respiratory function parameters within 3 weeks. It was also demonstrated that the post-PNX lung growth in mice involves not only a simple increase in alveolar size, but an increase in the number of alveoli (20).

In earlier studies of PNX models, quantitative morphological methods were employed (8, 15, 21); later, design-based stereology (22, 23) was more routinely available to characterize the structural changes after PNX (19, 20). All this quantification of compensatory lung growth, however, could only be accomplished after killing the animal and removing the lungs for histological analysis at a single time point. In addition, unless the entire lung undergoes exhaustive serial sectioning and analysis, assessment of differential lung growth cannot be fully determined, as tissue

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analysis is limited to the sections sampled. A noninvasive morphometric method would be extraordinarily useful for longitudinal studies to fully understand the time course of lung growth. Recently, this became possible through hyperpolarized ^3He magnetic resonance imaging (MRI). ^3He lung morphometry has been successfully implemented and validated in humans for many years (24–30), and was recently developed and validated in mice (31–33) and dogs (34). It can provide many of the same physiological parameters as in quantitative histology, but noninvasively and with tomographic information. This technique will be particularly powerful in longitudinally evaluating lung microstructural changes *in vivo* with advantages in time, accuracy, and reduced number of animals needed for serial experiments.

In this study, we employed ^3He MRI to image *in vivo* morphometry at baseline and to serially assess compensatory growth after left PNx in mice. The microstructural changes (alveolar-duct radius [R], alveolar depth [h], and mean linear intercept [L_m], among other parameters) after PNx were quantified and the time course of compensatory lung growth was evaluated. The formation of new alveoli and full restoration of the alveolar gas exchange area was assessed noninvasively. With this technique, we now have a noninvasive, *in vivo* method to serially assess the effectiveness of therapeutic interventions on post-PNx lung growth in the same mouse.

MATERIALS AND METHODS

Animal Preparation for MRI

All animal experiments were approved by the Washington University Animal Studies Committee. A total of 12-wk-old C57BL/6 mice were used: one group ($n = 6$) underwent left lung PNx; the other group ($n = 4$) was subjected to a sham operation (thoracotomy without tissue resection). Please refer to the online supplement for details. MRI was performed at baseline (immediately before PNx), 3 days, and 30 days after surgery.

Magnetic Resonance Imaging

^3He gas was hyperpolarized (nominally 40% polarization) with custom-built and commercial polarizers (GE Healthcare, Durham, NC) using spin-exchange optical pumping. MRI was performed using a doubly resonant solenoid coil (tuned to both ^3He and ^1H frequencies) on a Varian 4.7 T horizontal magnet (Oxford, UK). A trigger signal from the ventilator was used to control the image data acquisition during respiration. ^1H MRI was acquired with a two-dimensional gradient echo sequence; nine axial slices enabled the coverage of the entire lung (the center five slices nearly span the entire lung) and allowed anatomical orientation and volume measurement. A full acquisition required a number of breaths equal to the number of phase-encoding steps (all slices were acquired for one k-space value for each breath). ^1H MRI was performed twice during the same session: one acquisition during exhalation, the other at peak inspiration (“lower” and “higher” pressure, respectively). Volumes measured at two different pressures were used to calculate lung compliance. ^3He diffusion MRI images were acquired at the center-five slice positions of ^1H MRI using the same sequence as ^1H , with embedded

bipolar diffusion-sensitizing gradients. ^3He MRI was performed during breath hold at the peak of the respiratory cycle (peak pressure) to guarantee that image morphometry data were measured when the lung was fully inflated. ^1H and ^3He images were both acquired in a k-space interleaved fashion (see additional details in the online supplement).

Data Analysis

The area of lung parenchyma on each image was segmented by counting all voxels with intensities below a threshold in the lung region, which excluded large blood vessels automatically. The trachea and large conducting airway were manually excluded. The right lung was separated from the left by manual tracing. Lung volume was calculated by multiplying the sum of the area on each slice by the slice thickness. Compliance was calculated from the change in volume at two levels of pressure.

^3He MRI images of 6-b values are fitted to the theoretical model on a voxel-by-voxel basis using Bayesian analysis software (Washington University, St. Louis, MO). This generates parametric maps of R , h , L_m , the alveolar density, and the surface-to-volume ratio. Total alveolar number is the product of alveolar density and the lung volume measured by ^1H images. Total alveolar surface area was calculated using surface-to-volume ratio and the lung volume.

Statistical analysis is described in the online supplement.

Histology, Morphometry, and Quasistatic Compliance

Mice were killed at Day 30 and lungs harvested for tissue histology and morphometry.

Quasistatic compliance measurements were performed with the flexiVent system (SCIREQ, Montreal, PQ, Canada). Please refer to the online supplement for details.

RESULTS

Lung Volume

Representative ^1H MR images for a PNx mouse are shown in Figure 1, with a summary in Table 1. Removal of the left lung resulted in vacated space that was occupied by a shift of the right lung into the left hemithorax. In all the animals at baseline, the right lung accounted for roughly 65% of the total lung volume. For the PNx group, a striking increase of right lung volume ($\sim 30\%$) was observed at Day 3, with further expansion ($\sim 15\%$) up to Day 30; for the sham group, the right lung volume remained unchanged during the 30-day period (Figure 2).

Lung Compliance

The total lung compliance measured via MRI at 3 days after PNx was 0.026 ml/(cm H_2O) which decreased 14% compared with baseline; at 30 days after PNx, it increased above the level of baseline to be 0.034 ml/(cm H_2O) (Figure 3A), suggesting that the overall static mechanical properties were restored by Day 30. For the sham group, there was no effective change.

The quasistatic lung compliance measured by flexiVent decreased by 30% 5 days after PNx and returned to the baseline level by Day 12 (Figure 3B).

Figure 1. Representative ^1H magnetic resonance imaging (MRI) images (axial imaging plane) for mouse lungs at baseline (top), 3 days after pneumonectomy (post-PNx; middle), and 30 days after PNx (bottom; at baseline, both right and left lungs are visualized). After PNx, only the right lung (appearing on the left in the images) remains in the thorax with great expansion of the volume. The accessory lobe of the right lung is seen extending into the left hemithorax.

TABLE 1. SUMMARY OF MORPHOMETRIC PARAMETERS OBTAINED VIA ^3He MAGNETIC RESONANCE IMAGING FROM THE RIGHT LUNGS OF SIX PNEUMONECTOMY MICE AND FOUR SHAM MICE AT BASELINE AND AT DAYS 3 AND 30 AFTER PNEUMONECTOMY

	No. of Animals	Volume (mm^3)	R (μm)	h (μm)	Lm (μm)	Alveolar No.	Alveolar Surface Area (cm^2)
PNX, Day 0	6	585.4 $\pm$ 51.2	97.5 $\pm$ 2.5	48.8 $\pm$ 2	64.1 $\pm$ 4.7	2.2 $\times 10^6 \pm 1.4 \times 10^5$	376.6 $\pm$ 26.7
PNX, Day 3	6	844.3 $\pm$ 132.1*	100.8 $\pm$ 2.2*	46.5 $\pm$ 1.5*	69.9 $\pm$ 4.1*	2.8 $\times 10^6 \pm 2.8 \times 10^5$ *	492.6 $\pm$ 52.4*
PNX, Day 30	6	983.5 $\pm$ 142.7*†	100.1 $\pm$ 1.4*	48 $\pm$ 1.6†	67.7 $\pm$ 2.8*†	3.3 $\times 10^6 \pm 3.8 \times 10^5$ *†	593 $\pm$ 65.0*†
Sham, Day 0	4	620.1 $\pm$ 149	98.3 $\pm$ 3	48.1 $\pm$ 1.8	66 $\pm$ 5.3	2.2 $\times 10^6 \pm 3.5 \times 10^5$	384.5 $\pm$ 67.5
Sham, Day 3	4	593.4 $\pm$ 225.6	97.8 $\pm$ 2.1	48.5 $\pm$ 2.9	64.4 $\pm$ 5.2	2.1 $\times 10^6 \pm 3.7 \times 10^5$	375.6 $\pm$ 64.1
Sham, Day 30	4	616.6 $\pm$ 113.2	98.8 $\pm$ 2.5	48.7 $\pm$ 2.7	65.7 $\pm$ 5.4	2.1 $\times 10^6 \pm 3.0 \times 10^5$	382.8 $\pm$ 52.7

Definition of abbreviations: h , alveolar depth; Lm , mean linear intercept; PNX, pneumonectomy; R , acinar airway radius.

The table gives values of key parameters in our model. Lung volume measured by ^1H magnetic resonance imaging is also listed, as well as the calculated parameters, alveolar number and alveolar surface area. Note that the lung parameters (R , h , and Lm) between total lung and right lung differed by less than 1%.

* $P < 0.001$ versus sham at baseline and versus PNX at baseline.

† $P < 0.05$ versus PNX at Day 3.

Microstructural Parameters

Microstructural parameters R , h , and Lm of all mice are summarized in Table 1. Representative ^3He ventilation images and parametric maps from PNX groups at different time points are shown in Figure 4. There was no correlation between ventilation and individual microstructural parameters. Average Lm significantly increased by Day 3 ($\sim +10\%$ compared with baseline, $P = 0.0004$), and then decreased by Day 30 ($\sim -3\%$ compared with Day 3, $P = 0.03$) for the PNX group. The same trend was observed in R , with the opposite trend in h (expected with duct elongation). This indicates an initial size increase of existing alveoli due to stretch by volume expansion. By Day 30, however, the size of individual alveoli/ducts was reduced toward baseline.

Due to the longitudinal nature of our study, lungs could only be obtained for histological examination at Day 30, at the end of the study. Histologic Lm measurements on lungs mean ($\pm$ SD) at Day 30 were 64.8 ($\pm$ 9.8) μm for PNX and 63.9 ($\pm$ 8.9) μm for sham groups, which is consistent with the data obtained from our MRI measurements. It should be noted that the standard deviation for the Lm is much smaller in the MRI measurement than with the traditional histologic method, suggesting that the MRI measurement is more precise, with the bonus of spatially resolved information. This is consistent with our past imaging and validation studies (26, 27, 32, 35).

Total Alveolar Number

Although lung microstructure changed only modestly, lung volume grew significantly, and a remarkable increase in total alveolar number of the remaining lung was seen at Days 3 and 30 after PNX. There was a 30% gain in number of alveoli at Day 3 after PNX and then 60% at Day 30 after PNX, which achieves 100% of the total alveolar number of both lungs before PNX (Figure 5). This and Table 1 demonstrate that, during compensatory lung growth, there is not only an initial increase in alveolar size, but also significant growth of new alveoli.

Total Alveolar Surface Area

The total alveolar surface area of the right lung increased by 30% by Day 3 compared with baseline, and, by Day 30, reached the same level of both lungs at baseline (Figure 6). This demonstrates that compensatory lung growth resulted in an increase of the gas exchange area before Day 3 and full restoration by Day 30 in mice after PNX. This restoration was largely achieved by an increase in alveolar number.

DISCUSSION

This is the first *in vivo* quantitative, longitudinal assessment of compensatory lung growth after PNX in mice via ^1H and ^3He

MRI. Our results show an initial increase in alveolar size and complete restoration of the lung volume, the lung compliance, the total alveolar number, and the total alveolar surface area by compensatory lung growth in mice *in vivo*. This is consistent with prior histological studies conducted in different animals at various time points (19, 20). Compared with those studies, the main advantage of our imaging technique enables noninvasive longitudinal measurement in the same animals; ^3He morphometry is especially powerful in evaluating the dynamics of structural changes during compensatory lung growth after PNX.

Longitudinal study in the same animal by MRI provides better understanding of the time course and the mechanism of post-PNX compensatory lung growth. Immediately after PNX, MRI images showed that empty space was created within the thoracic cavity, which directly led to the expansion of the lung tissue. Quantitatively, on one hand, our results revealed that the volume normalization has almost been finished by 3 days after PNX. On the other hand, in the early stage after PNX, the acinar space expanded disproportionately with the total lung volume, which was reflected by the modest increase in Lm and R . This disproportionality can be explained by the simultaneous increase of total alveolar number, which had already begun within 3 days after surgery. The regeneration of alveoli continued after Day 3, whereas the expansion of the total lung volume slowed. This is consistent with the slight decrease of Lm and R by Day 30. The finding that the formation of new alveoli lags behind the volume expansion supports the hypothesis that the mechanical stress and strain is an important *in vivo* signal stimulating compensatory lung growth.

Figure 2. Right lung volume at baseline (pre-PNX), and at Days 3 and 30 after PNX. Each mouse's measurements were normalized by its own baseline measurement from both lungs. Data are presented as mean $\pm$ SD. * $P < 0.001$ versus sham right lung at baseline; § $P < 0.001$ versus PNX right lung at baseline; † $P < 0.05$ versus PNX at Day 3.

Figure 3. Total lung compliance before and after PNX. (A) Total lung compliance measured by MRI-based method at baseline (pre-PNX), and at Days 3 and 30 after PNX. Each mouse's measurements were normalized by its own baseline measurement. Note that, for the baseline of PNX group and all the time points of the Sham group, the total lung includes both right and left lungs, whereas for 3 days and 30 days of the PNX group, the total lung refers to the residual right lung. Data are presented as mean $\pm$ SD. (B) Total lung compliance determined by quasistatic measurements (flexiVent) at baseline (pre-PNX) and at Days 3 and 30 after PNX. Each mouse's measurements were normalized by its own baseline measurement. Data are presented as mean $\pm$ SD. * $P < 0.001$ versus Sham at baseline. $^{\S}P < 0.001$ versus PNX at baseline., $^{\dagger}P < 0.05$ versus PNX at Day 5.

Additional insights on mechanical properties of lung tissue are provided by compliance, determined here by the volume measurement at two pressures via ^1H MRI. The change of the total lung compliance is accordant with that of the total lung

volume, as expected. This leads us to postulate that synthesis or alteration of extracellular matrix had occurred during compensatory lung growth. We were wary of lung damage by longitudinal assessment of the same animals by the flexiVent device.

Figure 4. Representative MRI images and parameter maps. (A) Representative ^3He ventilation images of a PNX mouse, showing fairly homogeneous ventilation after PNX as baseline. (B) Representative mean linear intercept (L_m) map of a PNX mouse at baseline and at Days 3 and 30 after PNX from ^3He morphometry. The L_m increase at Day 3 shown in Table 1 is clearly demonstrated here, with obvious spatial variation. (C) Average L_m value for two regions of interest (ROIs). ROI 1 is located in the anterior area of the peripheral lung (solid arrow in [B]); ROI 2 is located in the accessory lobe of the right lung (dashed arrow in [B]).

Figure 5. Right lung alveolar number at baseline (pre-PNX) and at Days 3 and 30 after PNX. Each mouse's measurements were normalized by its own baseline measurement from both lungs. Data are presented as mean $\pm$ SD. * $P < 0.001$ versus sham right lung at baseline; § $P < 0.001$ versus PNX right lung at baseline; † $P < 0.05$ versus PNX at Day 3.

Direct physiologic assessment of lung compliance by flexiVent was conducted in two different groups of animals at a somewhat different set of time points after surgery; however, the mice were of the same strain, the same age, and were subjected to the same surgical procedure by the same surgeon. The trends of compliance change before and after PNX determined by these two measurements were similar: the total lung compliance first decreased after PNX and eventually returned to baseline levels, as shown in Figure 3.

An important, unique strength of ^3He morphometry is its ability to display the regional variation of lung microstructure by complete tissue sampling (each voxel is averaged over its volume) with relatively little effort compared with traditional stereological methods (27, 28, 30). Parameter maps of each individual mouse provide comprehensive regional variation of microstructure changes as a function of time. Because the fissures are not resolvable in our MRI images, currently we are not able to segment and precisely quantify lobar differences during the compensatory lung growth. However, based on the region-of-interest analysis at different parts of the lung, no consistent heterogeneous alveolar growth has been observed for the six PNX mice we studied, although interanimal variation was nontrivial. We also investigated the variation of microstructural parameters as a function of signal intensity of ^3He and found no correlation.

High-resolution computed tomography (CT) is another non-invasive approach that has been applied to the PNX model (36). It can be used to reconstruct lobar anatomy and quantify regional morphology of the lung parenchyma and conduction structure. The relative air and tissue/blood volume after lung resection can be quantified by using the CT attenuation value (37, 38). For comparison purposes only, we present data from our separate micro-CT study of age-matched C57BL/6 mice (12 wk) (see the online supplement for details). We found simultaneous increases in air and tissue/blood volume after PNX (Figure 7). We note that the last time point of this micro-CT study is earlier than that of our MRI study; this may explain why tissue/blood volume increased by 34% at Day 21, which is slightly smaller than the magnitude expected from a completed post-PNX regrowth. In any case, new alveolar growth definitely contributes to the tissue/blood volume increase. Moreover, perfusion to the remaining lung also increases after PNX, which is counted in our tissue/blood volume measurement in Figure 7. The tissue/blood volume and air volume for the sham group was approximately the same at different time points. It should be noted that high-resolution CT could not distinguish parenchymal tissue from microvascular blood, although this fact does not

detract from *in vivo* assessment of compensatory response via CT (39). One of the main disadvantages of CT is the radiation exposure, which makes longitudinal study difficult and potentially harmful; moreover, the current resolution limit precludes *in vivo* microstructure assessment. Compared with CT, MRI is radiation free and can precisely quantify the microstructure changes with regional information based on ^3He diffusion measurements.

Our technique is limited by a somewhat simplified lung model, which relies upon a few reasonable assumptions. First, we adopted the eight-alveolar model (eight alveoli around one alveolar duct), which is based upon stereological assessment (31, 40). Our method measures only two independent parameters (outer radius of acinar duct, R and alveolar depth, h , which are relatively insensitive to independent changes in alveolar length) (26). Second, we assume that, during the diffusion time ($2\Delta = 880 \mu\text{s}$, our shortest possible time given gradient-hardware restrictions), most ^3He atoms diffuse in a single acinar airway without escaping into adjoining airways. Finally, we simplify the distributions of acinar airway sizes into one average value per parameter; however, the ranges within the acinus are relatively small ($R = 70\text{--}100 \mu\text{m}$, $r = 40\text{--}75 \mu\text{m}$, and $h = 30\text{--}55 \mu\text{m}$) (31). We note that the precise mathematical model becomes invalid when tissue destruction is severe and the ducts less cylindrical, although our previous study demonstrated that ^3He lung morphometry still has excellent correlation with histology in patients with severe emphysema (26). The enlargement of acinar space in PNX models is modest, for which our technique is ideally suited.

Validation of ^3He lung morphometry against quantitative morphometry has been done in humans and mice. In humans, an excellent correlation between ^3He lung morphometry and direct L_m measurement was shown (correlation coefficient [R^2] = 0.985), and the difference between the two is much smaller than that between normal and emphysematous lungs (26). In healthy mice, the mean L_m , measured by ^3He lung morphometry, was $61 \mu\text{m}$ compared with $52 \mu\text{m}$ measured by histology in the same mice (32); in addition, this is consistent with L_m reported in healthy mice of other studies, which ranged from 44 to $63 \mu\text{m}$ (41). For PNX mice, the alveolar density of right lung of C57BL/6 mice 21 days after PNX was reported by histology to be 568.7 cm^{-3} (19) compared with 629.4 cm^{-3} measured in our study for right lung of mice 30 days after PNX. The alveolar density for PNX mice 30 days after PNX was $3,406 \text{ mm}^{-3}$ measured by ^3He lung morphometry in our study; Fehrenbach and colleagues showed by stereology that the alveolar density for mice 20 days

Figure 6. Right lung alveolar surface area at baseline (pre-PNX) and at Days 3 and 30 after PNX. Each mouse's measurements were normalized by its own baseline measurement from both lungs. Data are presented as mean $\pm$ SD. * $P < 0.001$ versus sham right lung at baseline; § $P < 0.001$ versus PNX right lung at baseline; † $P < 0.05$ versus PNX at Day 3.

Figure 7. Tissue and air volume of the right lung at baseline (pre-PNX) and at Days 0, 5, 12, and 21 after PNX. Note that this is from a different set of mice and at different time points from the MRI study. Data are presented as mean $\pm$ SD. * $P < 0.04$ versus sham right lung at baseline; § $P < 0.03$ versus PNX right lung at baseline.

after PNX was $2,112 \text{ mm}^{-3}$ (20). There are some systematic differences between ^3He lung morphometry and histology, but the differences are consistent and small, especially relative to the variation seen among different groups employing stereological methods (summarized in Ref. 31). Some of these differences can be attributed to different levels of lung inflation during *in vivo* MRI scan and stereological measurement, or to differential shrinkage during processing. Importantly, ^3He lung morphometry is volume averaged with data acquired from thousands of voxels, which provides complete tissue sampling, compared with the very thin two-dimensional samples of the three-dimensional structure with the histological approach. Nevertheless, in the PNX model, the relative change between parameters at different time points after PNX measured by ^3He morphometry and the histological results from the literature is comparable. For instance, we observed a full normalization of alveolar number in mice 30 days after PNX compared with results shown by histology of an approximately 82% restoration of alveolar number in mice 20 days after PNX (20). In summary, this study demonstrates that microstructural changes during post-PNX compensatory lung growth can be precisely quantified by hyperpolarized ^3He lung morphometry. The total lung volume, alveolar surface area, and alveolar number rapidly increased and were fully restored by 30 days after PNX in the mouse. Specifically, our findings show that compensatory lung growth in the murine model is achieved by both alveolar multiplication and growth in alveolar size. Lung compliance can be quantified by ^1H MRI alone; we found that the total lung compliance was also normalized by compensatory lung growth. We conclude that ^3He lung morphometry, with accompanying ^1H MRI, has great potential to be a valuable tool in understanding the time course and the mechanism of lung growth in individual animals, and may provide insight into human postnatal lung growth and lung regeneration.

Author disclosures are available with the text of this article at www.atsjournals.org.

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