HIGHLIGHTED TOPIC | Lung Growth and Repair

Smooth muscle development during postnatal growth of distal bronchioles in infant rhesus monkeys

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ONE OF THE PRINCIPAL HALLMARKS of allergic asthma is severe bronchioles in infant rhesus monkeys.

Smooth muscle development during postnatal growth of distal bronchioles in infant rhesus monkeys. J Appl Physiol 97: 2364–2371, 2004. First published September 3, 2004; doi:10.1152/japplphysiol.00476.2004.—Development of smooth muscle in conducting airways begins early in fetal life. Whereas the pattern and regulation of smooth muscle differentiation are well-defined, the impact of airway growth on the process is not. To evaluate the transformations in organization during postnatal growth, smooth muscle bundle organization (size, abundance, and orientation) was mapped in five generations of distal airways of infant rhesus monkeys (5 days and 1, 2, 3, and 6 mo old). On the basis of direct measurement of the bronchiole proximal to the terminal bronchiole, length increased by 2-fold, diameter by 1.35-fold, and surface area by 2.8-fold between 5 days and 6 mo of age. Smooth muscle bundle size was greater in proximal bronchioles than in respiratory bronchioles and did not change with age. However, relative bundle size decreased in proportion to airway size as the airways grew. Relative bundle abundance was constant regardless of airway generation or age. The distribution of smooth muscle bundle orientation changed with age in each airway generation, and there were significant changes in the terminal and respiratory bronchioles. We conclude that smooth muscle undergoes marked organizational changes as airways grow during postnatal development.

airway smooth muscle; postnatal lung development

thickening is an increase in smooth muscle mass, which is doubled compared with age-matched control subjects (1).

With lung maturation, lung function and airway reactivity change. Examples include the rate of emptying during forced expiration decreasing during infancy (31) and children’s airways being more reactive to methylcholine and histamine than adult airways (32). Smooth muscle has the potential to modulate airway function and reactivity, and this ability is most likely dependent on the amount, location, orientation, contractile properties, and length-tension relationship of the smooth muscle (36). Airway smooth muscle is present in the fetus and undergoes significant changes as part of pre- and postnatal lung development (5, 30). This study was designed to define the organizational changes in airway smooth muscle during postnatal morphogenesis because the etiology of allergic asthma is now thought to be a combination of genetics, the environment, and development (16). We compared smooth muscle bundle size, abundance, and orientation in infant monkeys during early postnatal lung development (5 days and 1, 2, 3, and 6 mo of age). The most distal three generations of nonalveolarized bronchioles and the most proximal two generations of respiratory bronchioles in nonhuman primates, rhesus monkeys, were examined. These generations were chosen because there is evidence that peripheral airways contribute to asthma (21) and because, in humans, terminal bronchioles are transformed into respiratory bronchioles during postnatal alveolarization (25).

Airways in the same lung segment, the caudal portion of the left cranial lobe, were evaluated in each animal to ensure that specimen selection was consistent. To define the pattern of changes in airway size during growth, we compared absolute length and diameter of the same airway generation (the bronchiole immediately proximal to the terminal bronchiole) in each animal.

MATERIALS AND METHODS

Animal and experimental protocol. All monkeys selected for these studies were California National Primate Research Center colony-born male rhesus macaques (Macaca mulatta). Care and housing of animals before, during, and after treatment complied with the provisions of the Institute of Laboratory Animal Resources and conformed to practices established by the American Association for Accreditation of Laboratory Animal Care.

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To determine the normal pattern of airway development in the rhesus monkey, three to six infants, housed from birth in chemical, biological, and radiological filtered air, were necropsied at each of the following ages: 5 days old (4–6 days), 1 mo old (25–27 days), 2 mo old (63–66 days), 3 mo old (85–88 days), and 6 mo old. These time points were chosen because they span a period of rapid lung growth in both the monkey and human (4, 14).

**Dissection and tissue evaluation.** Monkeys were euthanized with an overdose of pentobarbital sodium after being sedated with Telazol (8 mg/kg im) and anesthetized with Diprivan (0.1–0.2 mg·kg\(^{-1}\)·min\(^{-1}\) iv), with the dose adjusted as necessary by the attending veterinarian. The monkeys were then necropsied after exsanguination through the posterior vena cava. Body weight was taken at necropsy. The right middle lobe was cannulated separately and sanguination through the posterior vena cava. Body weight was taken at necropsy. The right middle lobe was cannulated separately and fixed by inflation with glutaraldehyde-paraformaldehyde (1–10%) at 30 cmH\(_2\)O pressure. After 4 h of fixation under pressure, lung volume was measured by fluid displacement. The left cranial lobe was fixed via the airways through a bronchial cannula with 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 30 cmH\(_2\)O pressure for 4 h. After fixation, the caudal segment of the left cranial lobe was dissected free, and the axial pathway (from segmental bronchi to alveolar ducts) and its distal side branches were exposed by microdissection into a costal and a mediastinal half (26, 27). The number of branches was determined by direct count of all branches beginning at the lobar bronchus and continuing along the axial path to the first proximal respiratory bronchiole. Smooth muscle orientation was defined for the costal half of the airways. Dissected lobes were permeabilized with 0.3% Triton-X 100, washed with 0.1 M PBS, incubated in 0.066 μM Alexa Fluor 568 phalloidin (Molecular Probes, Eugene, OR) for 20 min, and rinsed with PBS. One axial pathway with at least three of its distal side branches in the microdissected lungs of each animal were imaged using laser scanning confocal microscopy (Bio-Rad MRC 1024 ES mounted on an Olympus BX50WI microscope) with long working distance water-immersion objectives as described previously (28, 35). Alexa Fluor 568 phalloidin (excitation 578; emission 600) allowed visualization of airway smooth muscle because it stains filamentous actin, an abundant protein in smooth muscle cells. A smooth muscle bundle was identified as a group of transversely oriented smooth muscle cells stained with phalloidin and separated from each other by large spaces. Images for specific airway sites were collected using a 4× or 10× objective. Final magnification of all images used for measurements was 170×. Images were 20–40 μm apart in the z direction and had a depth of focus of 20 μm. Before analysis, images were tiled together to produce a composite map of the entire distal airway tree (Fig. 1). Airway sites evaluated (Fig. 1A) included the terminal bronchiole (TB); the next two most proximal airway generations, bronchiole 1 generation proximal (PB1) and bronchiole 2 generations proximal (PB2); and the first and second generations of respiratory bronchiole generation distal to the TB (RB1 and RB2, respectively). Respiratory bronchioles were identified during microdissection by the presence of alveolar outpockets. The exact position of the most proximal alveoli (RB1) in each branch path was confirmed on whole mounts using a nuclear dye (4′,6′-diamino-2-phenylindole) to identify epithelial cell distribution, and their positions were marked on the composite maps. The TB was defined as the airway generation proximal to RB1.

**Quantitation.** Stacks of images were used to measure the orientation of smooth muscle bundles, bundle profile size, and the number of smooth muscle bundles per airway length. Smooth muscle bundle orientation was measured as the angle of deviation (θ) from perpendicular to the axis of the airway segment (Fig. 1B). Zero degrees was perpendicular to the long axis of the airway, and 90° was parallel to the long axis. θ was measured for each smooth muscle bundle. Bundle angle mean and standard deviation were also calculated. Bundle abundance and profile size were determined by defining the boundaries of each airway segment on composite images. Three linear probes, oriented parallel to the airway axis, were superimposed over the image, and the number of intercepts of the probes with smooth muscle bundles was counted. Number per unit length of airway was calculated by dividing the total number of intercepts by the total length of the probes. Relative smooth muscle abundance was calculated as the number of smooth muscle bundles per 100 μm of airway length. The average size of each bundle was estimated as the mean of the absolute values for the length of the probe covering each bundle.

Fig. 1. A: composite map of laser scanning confocal images of smooth muscle in distal bronchioles of the rhesus monkey. Smooth muscle is identified with Alexa Fluor 568 phalloidin, a probe for filamentous actin. The following distal airways were evaluated in this study: PB2, bronchiole 2 generations proximal to the terminal bronchiole; PB1, bronchiole 1 generation proximal to the terminal bronchiole; TB, terminal bronchiole; RB1, first generation of respiratory bronchiole distal to the terminal bronchiole; RB2, second generation of respiratory bronchiole distal to the terminal bronchiole. Twenty-five images were used to make this map. B: diagram illustrating basis of measurement of smooth muscle bundle orientation in an airway. Arrow depicts the axis of airflow. Bundle angles (θ) were measured from a line perpendicular to the axis of the airway, at which θ = 0.
Bundle size was standardized to airway diameter size. The diameter of each airway was determined from direct measurement of airway maximum width, and the radius was calculated as width divided by two.

The length of airway generation PB1 (the bronchiole proximal to the TB) was determined as the absolute distance, parallel to the long axis, between the branch points proximal and distal to it, using images of both halves of the airway to define the exact branch point. The number of smooth muscle bundles was counted for this airway generation as well.

Statistics. All data are expressed as means ± SD. Differences between age groups and airway levels were determined by one-way ANOVA (SAS, SAS Institute, Cary, NC). Bundle size in relation to airway size was compared by regression analysis. Differences in slope and regression coefficient were compared between age groups (37). Distribution of smooth muscle bundle orientation was compared with 6-mo-old animals by χ² analysis (SAS, SAS Institute). *P < 0.05 was considered statistically significant.

RESULTS

5 days to 1 mo of age. Body weight and fixed lung volume of the right middle lobe did not change between 5 days and 1 mo of age (Fig. 2). During this time, the number of intrapulmonary airway branches in the axial pathway of the left cranial lobe (caudal segment) averaged 13, starting at the lobar bronchus and ending at the junction of the terminal and respiratory bronchioles (Fig. 3A). To establish how the dimensions of an airway change with age, we measured the length (Fig. 3B), diameter (Fig. 3C), and number of smooth muscle bundles (Fig. 3D) of one nonalveolarized bronchiole (PB1). These parameters remained the same between 5 days and 1 mo of age. This is reflected in the similar estimated surface area of PB1 at 5 days and 1 mo, 1,692,347 and 1,800,562 μm², respectively (Fig. 4). Smooth muscle size was similar in the five most distal bronchioles of 5-day-old and 1-mo-old monkeys (Table 1). However, there was a trend for 1-mo-old animals to have larger bundles per airway size than 5-day-old animals (Fig. 5). The relative abundance of smooth muscle bundles (number per 100

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**Fig. 2.** Body weight (A) and fixed lung volume (B) in infant rhesus monkeys at different ages after birth (5 days and 1, 2, 3, 6 mo). Values are means ± SD. *P < 0.05 compared with 6-mo-old animals.

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**Fig. 3.** Comparison of changes during bronchiolar growth in 5-day-old and 1-, 2-, 3-, and 6-mo-old rhesus monkeys. A: number of airway branches from the lobar bronchus to the first generation of respiratory bronchiole remained consistent with age. B: total length of 1 generation of nonalveolarized airway (PB1) increased with age. C: airway diameter of 1 generation of nonalveolarized airway (PB1) changed in size depending on the age when measured. D: absolute number of bundles in the PB1 got larger with postnatal age. Values are means ± SD. *P < 0.05 compared with 6-mo-old animals. #P < 0.05 compared with 3-mo-old animals.
μm of airway length) was similar in the distal bronchioles of 5-day-old and 1-mo-old animals (Table 2). The orientation of individual bundles was estimated by measuring the angle of deviation from perpendicular to the long axis of the airway (θ) (see Fig. 1B). Values of θ were tallied into four categories: 0–9.99°, 10–19.99°, 20–29.99°, and ≥30°. Categories were based on a study of smooth muscle orientation in the intraparenchymal airways of the cat and human by Lei et al. (22). There was a wide variation in the distribution of θ for smooth muscle bundles between distal generations and in the same airway generation between animals of the same and different ages (Fig. 6). There was a trend at 5 days and 1 mo for respiratory bronchioles to have a greater mean smooth muscle bundle θ than proximal bronchioles. At 5 days of age, the mean smooth muscle bundle θ was 9.8 ± 8.8° for PB2, 8.8 ± 8.2° for PB1, 10.3 ± 9.5° for TB, 11.5 ± 11.2° for RB1, and 11.0 ± 11.8° for RB2. At 1 mo of age, the mean θ was 8.3 ± 7.5° for PB2, 8.7 ± 7.2° for PB1, 6.4 ± 5.4° for TB, 9.9 ± 7.9° for RB1, and 14.6 ± 9.3° for RB2.

2–3 mo of age. Significant developmental changes in the distal airways and smooth muscle bundles occurred in infant rhesus monkeys between 2 and 3 mo of age. Their body weight doubled compared with 5-day-old and 1-mo-old animals (Fig. 2A). Lung volume increased as well; the fixed lung volume of the right middle lobe at 3 mo of age was ~1.7-fold greater than at 1 mo of age (Fig. 2B). The number of intrapulmonary airway branches in the axial pathway of the left cranial lobe averaged 13, unchanged from the earlier time points (Fig. 3A). On the other hand, the length of PB1 increased between 1 and 2 mo (Fig. 3B). Length then remained unchanged from 2 to 3 mo. In fact, the length of PB1 of 3-mo-old animals was significantly greater than 5-day-old and 1-mo-old animals (Fig. 3B). At 2 mo of age, the diameter of PB1 in infant animals was signif-

Table 1. Changes in size of smooth muscle bundles in distal bronchioles of infant rhesus monkeys during postnatal development

<table>
<thead>
<tr>
<th>Airway</th>
<th>Age</th>
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<th>Age</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
<td>1 mo</td>
<td>2 mo</td>
<td>3 mo</td>
</tr>
<tr>
<td>PB2</td>
<td>29.9 ± 5.5</td>
<td>32.6 ± 4.4</td>
<td>29.5 ± 5.0</td>
<td>30.1 ± 9.1</td>
</tr>
<tr>
<td>PB1</td>
<td>27.5 ± 5.3</td>
<td>29.3 ± 9.2</td>
<td>23.6 ± 2.7</td>
<td>31.1 ± 4.4</td>
</tr>
<tr>
<td>TB</td>
<td>25.7 ± 4.4</td>
<td>23.9 ± 5.1</td>
<td>24.2 ± 5.1</td>
<td>26.1 ± 4.8</td>
</tr>
<tr>
<td>RB1</td>
<td>27.6 ± 3.6</td>
<td>29.8 ± 10.2</td>
<td>14.0 ± 1.9*</td>
<td>25.4 ± 6.0</td>
</tr>
<tr>
<td>RB2</td>
<td>23.1 ± 2.9</td>
<td>23.4 ± 3.1</td>
<td>18.5 ± 2.4*</td>
<td>20.4 ± 7.6</td>
</tr>
</tbody>
</table>

Values are means ± SD given in μm. PB2, bronchiole 2 generations proximal to TB; PB1, bronchiole 1 generation proximal to TB; TB, terminal bronchiole; RB1, respiratory bronchiole 1 generation distal to TB; RB2, respiratory bronchiole 2 generations distal to TB. *P < 0.05 compared with PB2 for the same age group.

Fig. 4. Diagrammatic comparison of size changes in the PB of rhesus monkeys starting at 5 days of age and ending at 6 mo. Scaling for length (l) and diameter (d) is based on setting the value for 30-day-old animals equal to “1.” Luminal surface area (SA) was calculated from the values.

Fig. 5. Bundle size relative to airway size (radius in μm) for 5-day-old and for 1-, 2-, 3-, and 6-mo-old rhesus monkeys. There is a close correlation between the size of the airway and bundle size. Colored text describing the age of the rhesus monkeys, regression equation, and regression coefficient correlates to the colored lines on the graph. FA, filtered air. *P < 0.05 compared with 6-mo-old animals.
postnatal development

Table 2. Changes in relative abundance of smooth muscle bundles in distal bronchioles of infant rhesus monkeys during postnatal development

<table>
<thead>
<tr>
<th>Airway</th>
<th>5 days</th>
<th>1 mo</th>
<th>2 mo</th>
<th>3 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2</td>
<td>1.6±0.4</td>
<td>1.7±0.3</td>
<td>1.8±0.5</td>
<td>1.6±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>PB1</td>
<td>1.6±0.2</td>
<td>1.5±0.5</td>
<td>1.7±0.2</td>
<td>1.6±0.2</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>TB</td>
<td>1.7±0.3</td>
<td>1.8±0.1</td>
<td>1.9±0.5</td>
<td>1.5±0.3</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>RB1</td>
<td>1.5±0.0</td>
<td>1.6±0.2</td>
<td>2.1±0.0</td>
<td>1.3±0.3</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>RB2</td>
<td>1.5±0.5</td>
<td>1.9±0.3</td>
<td>1.8±0.1</td>
<td>1.6±0.3</td>
<td>1.4±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD given in no. per 100 μm of airway length. *P < 0.05 compared with PB2 for the same age group. †P < 0.05 compared with 6 mo of age for the same airway generation.

significantly decreased compared with the diameter at 3 mo of age (Fig. 3C). However, the estimated surface area of PB1 continued to enlarge with increasing age and length: the surface area was 2,053,185 μm² at 2 mo and 3,178,395 μm² at 3 mo (Fig. 4). Furthermore, the number of smooth muscle bundles around PB1 was increased at 2 and 3 mo compared with 5 and 1 mo (Fig. 3D). When the size of smooth muscle bundles in the distal bronchioles was compared between ages, there was no difference in bundle size for the first 3 mo of life (Table 1). However, 2-mo-old animals had significantly smaller bundles in the respiratory bronchioles compared with the proximal bronchioles (Table 1). The linear relationship between increasing airway size and increasing bundle size was retained at 2 and 3 mo of age (Fig. 5). However, with increasing age (at 3 mo), bundle size decreased in relation to airway size. The greatest change was in proximal bronchioles (largest radius). The relative abundance of smooth muscle bundles did not change in the five most distal bronchioles of 2- and 3-mo-old animals and was similar to 5-day-old and 1-mo-old animals (Table 2). However, 2-mo-old animals had significantly greater abundance in RB1 compared with PB2. The distribution of smooth muscle bundle θ varied between distal generations and in the same airway generation between animals of the same and different ages (Fig. 6). At 2 mo of age, the mean smooth muscle bundle θ was 9.0 ± 8.3° for PB2, 10.9 ± 8.8° for PB1, 12.6 ± 11.4° for TB, 10.7 ± 9.2° for RB1, and 10.1 ± 7.3° for RB2. At 3 mo of age, there was a trend for respiratory bronchioles to have a greater mean θ than more proximal bronchioles. The mean smooth muscle bundle θ was 9.4 ± 8.4° for PB2, 9.6 ± 8.2° for PB1, 9.3 ± 6.8° for TB, 10.5 ± 7.2° for RB1, and 14.0 ± 14.5° for RB2.

6 mo of age. By 6 mo of age, the juvenile monkeys had significant changes in overall growth of the airways and smooth muscle compared with all other time points. Body weight increased threefold in 6-mo-old animals compared with 5-day-old and 1-mo-old animals (P < 0.05) (Fig. 2A). Fixed lung volume of the right middle lobe was twofold greater in 6-mo-old animals compared with 5-day-old animals (P < 0.05) (Fig. 2B). The number of intrapulmonary airway branches in the axial pathway of the left cranial lobe from the lobar bronchus to the terminal and respiratory bronchioles still averaged 13 generations (Fig. 3A). PB1 grew in length between 3 and 6 mo of age (Fig. 3B). The length of this airway more than doubled between 5 days and 6 mo (P < 0.05). Airway diameter decreased early during postnatal growth and then increased by greater than one-third between 2 and 6 mo (Fig. 3C). Length and diameter changes resulted in a threefold increase in the total surface area of PB1 between 5 days and 6 mo of age (Fig. 4); the estimated surface area of PB1 at 6 mo of age was 5,090,025 μm². When adjusted for increases in
airway size, the absolute number of bundles in PB1 doubled between 5 days and 6 mo of age (\( P < 0.05 \)) (Fig. 3D). This increase was directly correlated to changes in airway length but not in diameter. Bundle size for a particular airway generation did not change significantly with increasing age in any of the airway generations evaluated (Table 1). However, 6-mo-old animals had significantly smaller bundles in RB2 compared with the PB2 (Table 1). The linear relationship between increasing airway size and increasing bundle size was retained at 6 mo of age (Fig. 5), but animals had significantly smaller bundle size in relation to airway size compared with all ages, especially in the larger bronchioles. The relative abundance of smooth muscle bundles was similar in the five most distal bronchioles of 6-mo-old animals (Table 2). Nevertheless, the abundance in respiratory bronchioles at 6 mo of age was significantly smaller compared with the abundance in respiratory bronchioles at 1 and 2 mo of age. The distribution of smooth muscle bundle \( \theta \) (Fig. 6) was significantly changed in the smaller bronchioles at 6 mo of age compared with earlier time points; distributional differences were found in the TB (at 1 mo), RB1 (at 1, 2, and 3 mo), and RB2 (at 5 days and 2 mo). Similar to prior ages, the respiratory bronchioles had a greater mean \( \theta \) than more proximal bronchioles at 6 mo of age. The mean smooth muscle bundle \( \theta \) was 10.3 \( \pm \) 9.4\( ^\circ \) for PB2, 9.3 \( \pm \) 8.1\( ^\circ \) for PB1, 9.7 \( \pm \) 8.0\( ^\circ \) for TB, 15.2 \( \pm \) 12.6\( ^\circ \) for RB1, and 13.3 \( \pm \) 10.6\( ^\circ \) for RB2.

DISCUSSION

The purpose of this study was to establish whether the organization of smooth muscle bundles in the walls of conducting airways changes as the lung grows in the early postnatal period. The time frame for postnatal lung development evaluated in this study is reasonably comparable to that of humans on the basis of alveolarization, the parameter best documented in human and macaque monkeys. The addition of new alveoli to the lungs of humans ceases between 2 and 3 yr of age (4, 23). For rhesus macaques, alveolarization ceases by 28 mo (17). The process by which the airways grow as the total lung volume increases and the associated changes in the organization of smooth muscle bundles in five contiguous generations of distal airways were evaluated for the first 6 mo of postnatal life. We chose to focus on the distal airways (bronchioles) because they are important contributors to resistance for the first 5 yr of life (15). There is also physiological and pathological evidence that they play a role in asthma [see Kraft (21) for review].

We evaluated smooth muscle bundles in three-dimensional samples rather than two-dimensional sections, which facilitated evaluation of three parameters: 1) the size of individual bundles, 2) the number of bundles per unit airway length, and 3) the orientation of bundles. These parameters were compared on the basis of airway generation, airway size, and age. We conclude that smooth muscle undergoes marked organizational changes as the airways grow. Distal airway maturation during the first 6 mo after birth involved more than doubling in length, increasing one-third in diameter, and increasing almost threefold in total surface area. During this time, relative smooth muscle abundance was constant regardless of airway generation or age. Because of the intrinsic variability in airway diameter of the same airway generation, we evaluated smooth muscle bundle size using two methods: 1) by airway generation and 2) in relation to airway size (diameter). When comparisons were done on an airway generation basis, we found that smooth muscle bundle size was greater in proximal bronchioles than respiratory bronchioles and that it did not change with age. However, when comparisons were done on an airway size basis, we found that there was a positive correlation between airway size and smooth muscle size at all ages. Yet, relative bundle size decreased in proportion to airway size as the animals aged. The distribution of smooth muscle bundle orientation changed with age in each airway generation, and there were significant changes in the terminal and respiratory bronchioles.

Previous studies have suggested that there is an airway level-specific heterogeneity in smooth muscle abundance that might be altered by a number of factors, including development (18). To ensure that we were comparing the same airway generations in different-aged animals, we used carefully defined specimens (26, 27). We identified the most distal nonalveolarized bronchiole by its junction with alveolarized (respiratory) bronchioles and established its position within the airway tree on the basis of the number of branch points down the axial path between this junction and the lobar bronchus. To further ensure that specimen selection did not bias the study, all of the airways were evaluated from the same portion of the same lobe in all animals. To avoid the potential problems of determining airway size produced by variable states of contractility during fixation, we used a standard fixation and relied on measurements of internal dimension identified by previous studies of smooth muscle function as a reliable measurement of airway size (19). This approach to evaluation allowed us to compare airways of different sizes with known generations and used the analytic approach previously employed to establish whether increases in smooth muscle abundance, or mass, may be contributing to airway reactivity in distal airways (8, 11, 33). We have established that the development of the smooth muscle in the airways differs depending on position within the airway tree and age.

The smooth muscle bundles in the distal airways of infant rhesus monkeys had a wide range of orientation, on the basis of \( \theta \), which changed with airway generation and age. Our laboratory has previously found an equally wide variation in orientation in older monkeys and in adult rats and rabbits (29), and other studies using adult animals have documented this as well (9, 22). The most significant changes in the distribution of smooth muscle orientation were found in the terminal and respiratory bronchioles of rhesus monkeys as they aged. Perhaps these changes reflect ongoing alveolarization. By 6 mo of age, rhesus monkeys had an average \( \theta \) that ranged from 9 to 15\( ^\circ \) depending on airway generation. This is relatively similar to the overall average (13\( ^\circ \)) calculated for adult humans and cats (9, 22).

Theoretical estimates suggest a strong impact of smooth muscle orientation (i.e., helical pitch) on airway contractility (2). The closer the orientation of a bundle to perpendicular (smaller \( \theta \), the greater degree of airway constriction for the amount of smooth muscle shortening. As the percentage of the bundles with greater helical pitch (higher \( \theta \)) increases, their contribution to the effective force of the entire smooth muscle bundle population operating on an individual airway segment decreases. The inverse is true when more of the bundles are
oriented closer to perpendicular (lower $\theta$). Thus airway resistance would be affected by smooth muscle orientation. Increases in airway wall stiffness expected from the wall thickening in airways of asthmatic individuals could alter the effect of bundle orientation on airway contractility, depending on the degree of airway wall stiffness and the directionality of the stiffness (2). Longitudinally stiff airways would be more responsive to smooth muscle bundles at smaller deviations from perpendicular, whereas circumferentially stiff airways would be more responsive to bundles at greater angles. Our study suggests that the extent of airway contractility and airway resistance in relation to smooth muscle bundle constriction varies greatly with postnatal age because bundle orientation changes during this time.

In summary, our study shows that postnatal maturation of conducting airways during growth seems to be regulated by at least two processes that modulate smooth muscle morphogenesis: one that establishes smooth muscle mass by regulating bundle size and abundance and the other that establishes bundle orientation. Distal airways do not appear to increase in size uniformly in all dimensions as the lung grows, which may contribute to the modulation of these two morphogenetic processes. Furthermore, the timing and pattern of these processes in distal bronchioles are unique to the airway level and dependent on whether the airway undergoes alveolarization to become respiratory bronchioles. As a consequence, the size of smooth muscle bundles is greatest in more proximal, nonalveolarized bronchioles and decreases with age during postnatal growth. Whereas potential regulatory processes that drive smooth muscle morphogenesis are poorly understood, especially under conditions of early postnatal exposure to allergens, our study defines the pattern and time course of events and identifies potential targets for disruption.

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