

Smooth muscle hypertrophy in distal airways of sensitized infant rhesus monkeys exposed to house dust mite allergen

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Summary

Background Airway smooth muscle hypertrophy is closely associated with the pathophysiology of hyper-reactive airways in allergic asthma.

Objective To determine whether repeated exposure to allergens during postnatal lung development promotes remodelling of airway smooth muscle.

Methods Infant, male rhesus monkeys (30-day-old) were sensitized to house dust mite allergen (HDMA) and then exposed to HDMA aerosol periodically over 5 months. Smooth muscle mass and bundle size and abundance in conducting airways were measured and compared with age-matched control (filtered air-exposed) monkeys.

Results Total smooth muscle mass and average bundle size were significantly greater in the conducting airways of monkeys exposed to HDMA. Smooth muscle bundle abundance was not affected by exposure to HDMA.

Conclusion Repeated cycles of allergen exposure alter postnatal morphogenesis of smooth muscle, affecting both total mass and bundle size, in conducting airways of infant monkeys.

Keywords airway smooth muscle, asthma, postnatal lung development, rhesus monkey

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Introduction

Severe reactive bronchospasm is one of the principal hallmarks of allergic asthma and is often associated with persistent airway hyper-reactivity or incompletely reversible airway obstruction [1, 2]. While the exact role airway smooth muscle plays in abnormal airway responsiveness is not clear [3–5], an increase in the amount of smooth muscle is one of the major contributors to the airway wall thickening characteristic in the lungs of chronic asthmatics [4, 6, 7]. This appears to be the case for the central and peripheral airways in fatal asthma [8–10] as well as in non-fatal asthma [11–13]. Smooth muscle is present in fetal airways [14, 15] and undergoes significant changes as part of pre- and postnatal lung development [16–18]. Smooth muscle is the only significant contributor to airway thickening in young asthmatics, and the amount is doubled compared with age matched control subjects [10]. However, there is no experimental evidence establishing whether exposure of infants to

allergens during postnatal lung development will remodel airway smooth muscle.

We have developed a non-human primate model of allergic asthma using a known human allergen, house dust mite (*Dermatophagoides farinae*) allergen (HDMA), in adult and infant rhesus monkeys [19, 20]. Infant monkeys repeatedly exposed to HDMA have a significant increase in the eosinophil content of the central and terminal airways and in mucous cells in central airways [20], hallmarks of an allergic state. This study was designed to define the organizational changes in airway smooth muscle during postnatal morphogenesis using the same HDMA model in rhesus infants; this will establish whether exposure to airborne allergens during postnatal lung development alters smooth muscle organization. Our hypothesis was that smooth muscle remodelling associated with asthma begins with the disruption of normal developmental processes by exposure to allergens early in postnatal life when the lungs are undergoing active growth and differentiation. Total smooth muscle mass, individual bundle size, and abundance were compared in 6-month-old rhesus monkeys raised in either filtered air (FA) or exposed to HDMA periodically from 30 days of age for 5 months. Central and peripheral airways were evaluated because they are thought to be important contributors to allergic reactive airway diseases such as asthma [21, 22].

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Methods

Animal and experimental protocol

Twelve male infant rhesus monkeys (*Macaca mulatta*) were removed from their mothers at birth and raised as social groups in housing supplied with chemical, biological and radiological FA as previously described [20]. All monkeys were California National Primate Research Center (Davis, USA) colony-born rhesus macaques. Care and housing of animals complied with ILAR provisions and established American Association for Accreditation of Laboratory Animal Care practices. Six monkeys were sensitized to HDMA (*D. farinae*) at age 14 and 28 days, by subcutaneous inoculation of HDMA (10 mg) in alum and i.m. injection of heat-killed *Bordetella pertussis* cells (2.5×10^{11}). Sensitization was confirmed by skin testing with subcutaneous HDMA. Sensitized monkeys were exposed to HDMA aerosol for 11 episodes [23]. HDMA (Greer Laboratories Inc., Lenoir, NC, USA) was diluted in phosphate-buffered saline (PBS), the major ingredient of which was NaCl at 8.5 g/L and nebulized with a high-flow rate nebulizer (HEART; Westmed Inc., Tucson, AZ, USA) immersed in an ice-water bath during operation to reduce water evaporation from the solution. Polydispersed droplets with a volume median diameter of $\sim 2 \mu\text{m}$ were produced in an air stream of 19.9 L/min from the nebulizer. The aerosol was mixed with the inlet air stream of a 4.2 m^3 volume exposure chamber [24] to yield an aerosol of dry particles composed of allergen and salt residues. The HDMA particles had a mass median aerodynamic diameter of $1.29 \pm 0.12 \mu\text{m}$ with a geometric standard deviation (SD) of 2.12 ± 0.26 for the fitted log normal distributions. Values are mean $\pm$ SD for $n = 11$ size measurements. Six control infants were maintained in FA rather than an aerosol generated from PBS because a PBS aerosol does not have the equivalent particle size or total mass concentration of the HDMA aerosol; both properties are significantly smaller than the HDMA containing particles. Furthermore, a saline (PBS) aerosol was considered to be innocuous, much like that occurring at the sea shore. For a more detailed account of the preparation and characterization of the HDMA aerosol see [19, 20]. The 11 episodes of HDMA exposure consisted of 3 days of HDMA exposure (2 h/day on days 1–3 of each 14-day cycle). Detailed analysis of pulmonary function changes (airway resistance and hyper-responsiveness), immune responses, and basement membrane changes have been reported for the same group of monkeys [20, 25].

Dissection and tissue evaluation

All animals were killed at 6 months of age. Following exposure, monkeys were killed with an overdose of pentobarbital sodium after being sedated with telazol (8 mg/kg i.m.) and anesthetized with diprivan (0.1–0.2 mg/kg/min, i.v.) with the dose adjusted as necessary by the attending veterinarian. The monkeys were then necropsied following exsanguinations, which occurred through the posterior vena cava. The left cranial lobe was immediately fixed with 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 30 cm of pressure for 4 h via cannulation of the lobar bronchus for confocal microscopy. The right middle lobe was fixed with 1% glutaraldehyde/1% paraformaldehyde at 30 cm of pres-

sure for 2 h via cannulation of the lobar bronchus for high-resolution light microscopy. Fixation procedures were chosen based on our and other previous studies [14, 15, 26], which demonstrated that fixation by airway inflation maintains smooth muscle orientation.

Microdissection and whole mount preparation

After fixation, the caudal segment of the left cranial lobe was dissected free and glued to a coverslip by its costal surface using Nexaband S/C veterinary adhesive (Veterinary Products, Phoenix, AZ, USA). The costal half of the airway was defined as the portion of the airway wall parallel to the costal surface of the lung lobe. The axial pathway (from lobar bronchus to alveolar ducts) and at least three distal side branches were exposed by microdissection for whole mount viewing. The distal side branches included the terminal bronchiole (TB); the next two most proximal airway generations, proximal bronchiole generation 1 (PG1) and proximal bronchiole generation 2 (PG2) and the first and second generations of respiratory bronchioles (RB) distal to the TB (RB1 and RB2, respectively) (See Fig. 1). The exact position of RB1 (having the most proximal alveoli) in each

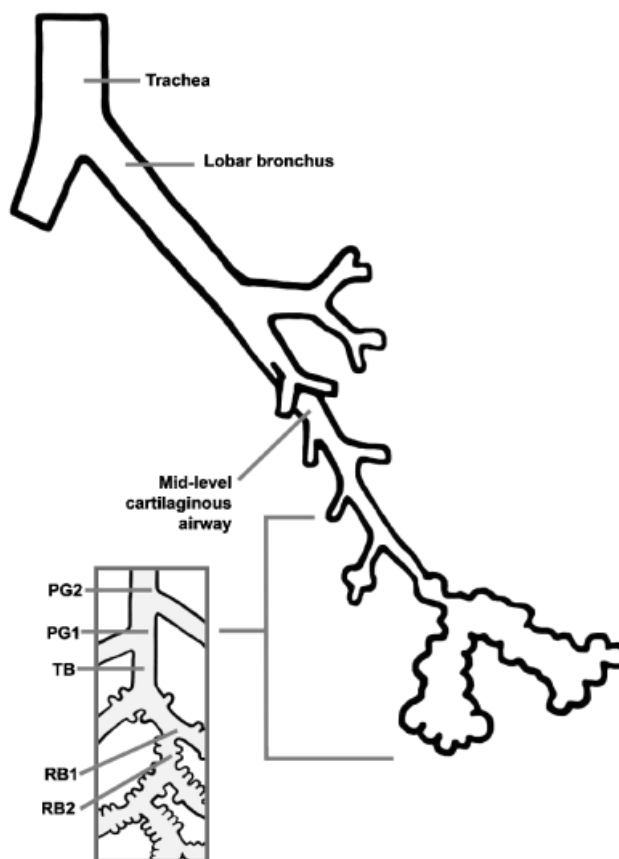


Fig. 1. Diagram of rhesus monkey airway tree. The axial pathway, from the lobar bronchus to the junction of the respiratory bronchioles, was microdissected. Smooth muscle mass was measured in mid-level cartilaginous airway generations 7 and 8. Smooth muscle bundle size and abundance were measured in five different distal airway generations: the terminal bronchiole (TB); the next two proximal airway generations, proximal bronchiole generation 1 (PG1) and proximal bronchiole generation 2 (PG2); and the first and second generations of respiratory bronchioles (RBs) distal to the TB (RB1 and RB2, respectively).

branch path was confirmed on whole mounts using the nuclear dye DAPI and UV epifluorescence microscopy (Leica MZFLIII, Solms, Germany); the transition from cuboidal epithelium to alveolar epithelium was apparent because of changes in the spacing of the nuclei. The TB was defined as the airway generation proximal to RB1. To control for differential HDMA aerosol deposition patterns, the same exact airway segments were evaluated in each animal.

Confocal microscopy

To visualize smooth muscle bundles via confocal microscopy, the microdissected lobes were incubated with fluorochrome-conjugated phalloidin, a probe for polymerized actin. A smooth muscle bundle was identified as a group of longitudinally oriented smooth muscle cells stained with phalloidin and separated from each other by large spaces. The tissue was permeabilized with 0.3% Triton-X 100, washed with PBS, incubated in 0.066 μM Alexa Fluor 568 phalloidin (Molecular Probes, Eugene, OR, USA) for 20 min, and washed with PBS. Distal airway generations were imaged using laser scanning confocal microscopy [Bio-Rad (Hercules, CA, USA) MRC 1024 ES mounted on an Olympus (Melville, NY, USA) BX50WI microscope] as described previously [26]. Briefly, a $\times 10$ long working distance water-immersion objective was used, and a series of images were taken through each airway generation at focal planes that were 20–40 μm apart with a focus depth of 20 μm . Images were stacked together to produce three-dimensional composites of specific areas of the airway tree (Figs 2c–f). The final magnification used for measuring was $\times 170$.

Airway architecture

The number of airway generations in the axial pathway from the lobar bronchus of the left cranial lobe (caudal segment) to the junction of the respiratory bronchioles was counted to establish that FA- and HDMA-exposed animals had equivalent airway generations. In addition, the length, and diameter for one non-alveolarized bronchiole (PG1) was compared for three animals per treatment group. The length 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. Internal airway size was determined by direct measurement of the maximum distance of the lumen perpendicular to the long axis. The number of smooth muscle bundles within PG1 was counted in both FA, and HDMA animals, and compared as well.

Smooth muscle mass (volume per surface)

For morphometric evaluation of smooth muscle mass, the right middle lobe was microdissected as described above. A block of tissue was cut perpendicular to the long axis of each airway generation along the axial path, and embedded as a large block in araldite [27]. The block evaluated contained mid-level cartilaginous airway generation 7 or 8 (Fig. 1). One-micrometre-thick sections were cut with glass knives, and stained with a solution of 1% toluidine blue for high-resolution light microscopy. Four fields from each block were selected by a uniform random sampling scheme, and imaged

at a final magnification of $\times 1000$. Smooth muscle mass (volume per unit surface area of basement membrane; $\mu\text{m}^3/\mu\text{m}^2$) was estimated by point and intercept counting as previously described [28]. Data from all four fields were used to calculate the mass of smooth muscle for each of the 12 animals counted (six FA and six HDMA).

Quantitation of smooth muscle bundles

Three-dimensional composites from three FA- and three HDMA-exposed monkeys having the largest changes in smooth muscle mass were used for direct measurements of smooth muscle bundle size and abundance. Size and abundance was determined by defining the boundaries of each airway generation on the same composite images. The entire airway of each generation, between the proximal branch point and the distal branch point, was measured. Three linear probes, which conformed to the curvature of the airway, were oriented parallel to the long airway axis and evenly superimposed over the image. A minimum of 50 bundles per airway generation per animal was measured. The average width (size) of each bundle was calculated as the mean length of the three probes covering each bundle. Smooth muscle bundle abundance was calculated as the mean number of smooth muscle bundles per 100 μm of airway length; the total number of intercepts per probe was divided by probe length. Bundle size and abundance were also standardized to airway diameter. Internal airway size was determined by direct measurement of the maximum distance of the lumen perpendicular to the long axis.

Statistics

All data were expressed as mean ± 1 SD. Differences between treatment groups and airway levels were determined by one-way ANOVA (SAS; SAS Institute Inc., Cary, NC, USA). Bundle size and abundance in relation to airway size was compared by regression analysis and differences in elevation and regression coefficient was compared between age and treatment groups [29]. $P < 0.05$ was considered statistically significant.

Results

Effect of house dust mite allergen on airway architecture

Inhalation of HDMA for 5 months did not alter airway architecture. The number of airway branches in the axial pathway from the lobar bronchus of the left cranial lobe (caudal segment) to the junction of the first RB1 averaged 13 generations for both FA- and HDMA-exposed monkeys. When one non-alveolarized bronchiole (PG1) was compared, there was no difference in airway length ($1565 \pm 174 \mu\text{m}$ FA vs. $1565 \pm 329 \mu\text{m}$ HDMA), diameter ($1035 \pm 41 \mu\text{m}$ FA vs. $1074 \pm 127 \mu\text{m}$ HDMA), and total number of bundles (25 ± 5 FA vs. 22 ± 6 HDMA) between FA- and HDMA-exposed animals.

Smooth muscle mass (volume per surface area)

Initial observation of mid-level cartilaginous airways revealed larger bundles of smooth muscle in the HDMA monkeys

compared with the FA monkeys (Figs 2a and b). When the mean smooth muscle mass (volume per unit surface area of basement membrane) was calculated for these airways, the HDMA-exposed animals had significantly more (60%) mass than animals exposed to FA (HDMA: $17.64 \pm 3.55 \mu\text{m}^3/\mu\text{m}^2$ vs. FA: $11.03 \pm 2.54 \mu\text{m}^3/\mu\text{m}^2$) (Fig. 3). As previously reported [20], group mean baseline airway resistance in the HDMA animals was elevated 65%, and airway responsiveness to histamine was reduced, but neither difference was statistically significant.

Size of individual bundles

In the distal airways of infant monkeys, smooth muscle bundle width was significantly thicker in proximal bronchioles than in respiratory bronchioles in both FA- and HDMA-exposed animals. (Table 1; Figs 2c and d vs. Figs 2e and f). HDMA-exposed monkeys had significantly larger

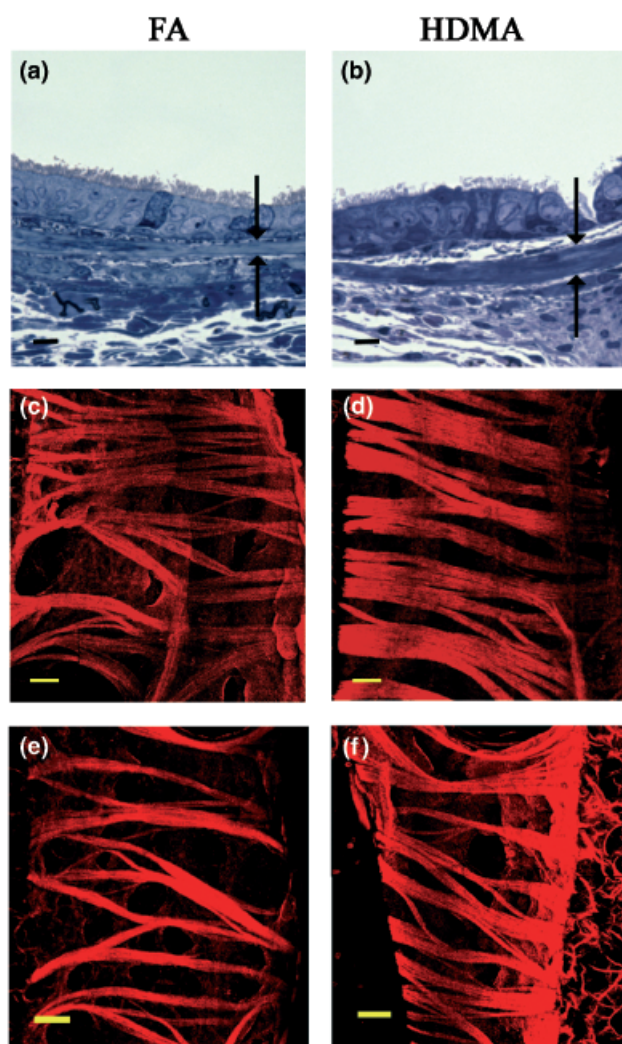


Fig. 2. Comparison of smooth muscle bundles in 6-month-old rhesus monkeys exposed to filtered air (FA) (a, c, e) or house dust mite allergen (HDMA) (b, d, e). Smooth muscle bundle width (between arrows) was greater in mid-level cartilaginous airway generation 7 or 8 of HDMA animals (b) compared with FA (a) animals. Magnification bar equals 10 μm . Smooth muscle bundles stained with AlexaFluor 568 phalloidin in laser confocal images in PG1 (a non-alveolarized bronchiole) (c, d), and RB1 (a alveolarized bronchiole) (e, f). Magnification bar equals 100 μm .

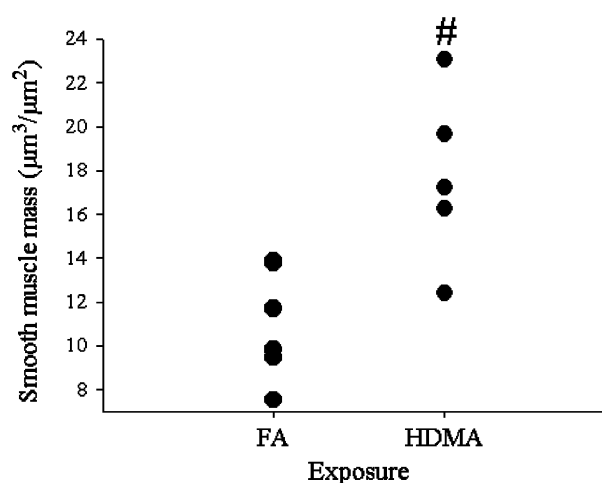


Fig. 3. Morphometric comparison of smooth muscle mass (volume per unit surface area of basement membrane) in mid-level cartilaginous airways (generation 7 or 8) between filtered air (FA) and house dust mite allergen (HDMA)-exposed 6-month old rhesus monkey revealed that HDMA animals had significantly more smooth muscle mass. # $P < 0.05$ compared with FA. Points represent individual monkeys. Although six monkeys were sampled per exposure group, two monkeys per exposure group had similar smooth muscle mass values (FA: 9.45 and 9.82 $\mu\text{m}^3/\mu\text{m}^2$, HDMA: 17.20 and 17.22 $\mu\text{m}^3/\mu\text{m}^2$) making the graph appear to have only 5 points per exposure group.

Table 1. Comparison of changes in size (μm) of smooth muscle bundles in distal bronchioles of 6-month-old infant rhesus monkeys following 5 months postnatal exposure to FA or allergen (HDMA)

Airway generation	Exposure FA	HDMA
PG2	29.7 ± 1.2	$42.5 \pm 3.7^{**}$
PG1	31.1 ± 5.1	$41.4 \pm 2.8^{**}$
TB	27.6 ± 6.5	35.7 ± 5.6
RB1	$27.5 \pm 4.6^*$	$24.8 \pm 6.2^*$
RB2	$23.0 \pm 1.8^*$	$25.7 \pm 5.6^*$

* $P < 0.05$ compared with PG2 for the same treatment group.

** $P < 0.05$ compared with FA for the same airway generation.

PG2, bronchiole 2 generations proximal to TB; PG1, 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; HDMA, house dust mite allergen; FA, filtered air.

smooth muscle bundles in the proximal bronchioles compared with FA-exposed monkeys (Table 1, Figs 2c and d). The average bundle size was larger in the terminal bronchioles of HDMA-exposed animals, but this difference was not statistically significant. When bundle size was standardized to airway diameter, the slope of this relationship was significantly steeper in HDMA-exposed monkeys compared with 6-month-old FA-exposed monkeys (Fig. 4a).

Abundance of bundles

There was no difference in the abundance of smooth muscle bundles (number per unit length of airway wall) in any of the airway generations evaluated in HDMA-exposed monkeys when compared with FA-exposed monkeys (Table 2). This was also true when relative abundance was standardized to airway diameter (Fig. 4b).

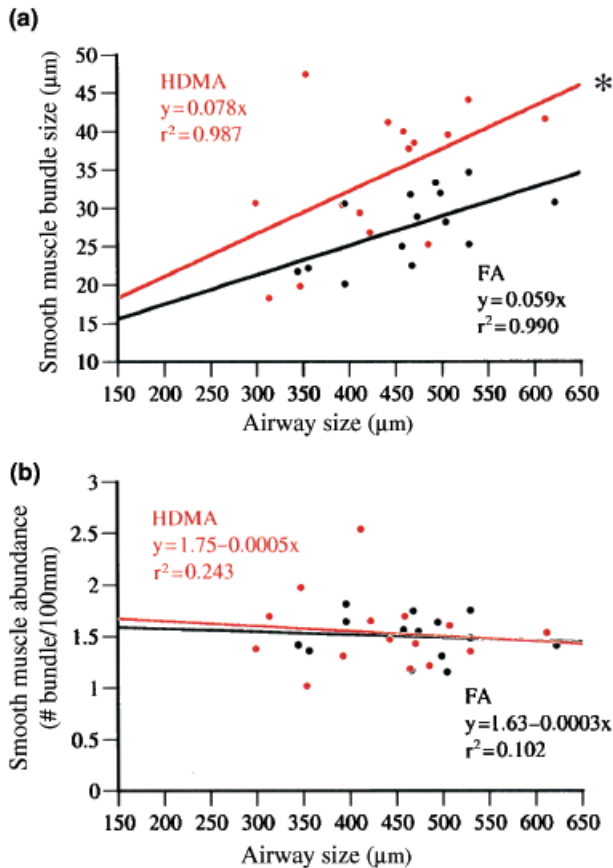


Fig. 4. (a) Bundle size relative to airway internal diameter in 6-month-old rhesus monkeys exposed to either filtered air (FA) (black) or house dust mite allergen (HDMA) (red) for 5 months. The slope of this relationship was significantly steeper in HDMA-exposed animals compared with FA-exposed monkeys. (b) Bundle abundance relative to airway internal diameter in 6-month-old rhesus monkeys exposed to FA (black) or HDMA (red) for 5 months. There was no difference between FA- and HDMA-exposed monkeys. * $P < 0.05$ compared with FA-exposed animals.

Table 2. Comparison of changes in relative abundance (# per 100 μm) of smooth muscle bundles in distal bronchioles of 6-month-old infant rhesus monkeys following 5 months postnatal exposure to FA or allergen (HDMA)

Airway generation	Exposure FA	HDMA
PG2	1.59 ± 0.1	1.54 ± 0.1
PG1	1.62 ± 0.1	1.44 ± 0.1
TB	1.51 ± 0.3	1.80 ± 0.7
RB1	1.35 ± 0.3	1.43 ± 0.3
RB2	1.44 ± 0.1	1.64 ± 0.3

PG2, bronchiole 2 generations proximal to TB; PG1, 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; HDMA, house dust mite allergen; FA, filtered air.

Discussion

A hallmark of airway remodelling in chronic allergic diseases such as asthma is a marked thickening of the airway wall [3–5]. The thickening encompasses a number of factors including

airway wall oedema, increased thickness of the connective tissue elements, and increases in the smooth muscle layer itself. While all of these changes may contribute to the airway narrowing associated with asthma, an increase in the mass of smooth muscle is considered likely to be the most important contributing factor to airway wall thickening in remodelled airways [4, 30]. In human asthmatics, there is a marked increase in smooth muscle mass in distal bronchioles that is closely related to the diameter of the airway [31]. In young patients dying of *status asthmaticus*, the increase in airway wall thickness is due primarily to an increase in the amount of smooth muscle as compared with older individuals with fatal asthma, in which all of the wall components increase [10].

A recent study concludes that persistent asthma (beginning in childhood and continuing into adulthood) is associated with allergy to HDMs, and relapse after remission is associated with allergy to HDMs [32]. We hypothesized that smooth muscle remodelling associated with asthma begins with the disruption of normal developmental processes by exposure to allergens early in postnatal life when the lungs are undergoing active growth and differentiation. To insure that we were comparing the same airway generations between treatment groups, airways that were evaluated came from the same portion of the same lung lobe, and the airway generations were counted starting from the lobar bronchus down to the respiratory bronchioles. This allowed us to define changes in smooth muscle mass and bundle size and abundance on a per generation basis and compare between generations in the same animal. A standard fixation procedure was used to avoid potential problems during quantization of mass, size, and abundance produced by variable states of smooth muscle contractility during fixation.

We found that infant monkeys exposed under controlled conditions to a known human allergen, HDMA, have increases in smooth muscle mass in the central (mid-level) airways and smooth muscle bundle size in the non-alveolarized distal airways. The increase in mass (60%) was greater, but in the same range as observed in mice when ovalbumin was used as an allergen (50% increase in mass) [33]. A close correlation between the diameter of the airway and the size of the bundles was found in all five distal airway generations evaluated; bundle size was larger in relation to airway size in animals exposed to HDMA at 6 months of age. However, HDMA exposure did not change the number of smooth muscle bundles, and the number was not greater in relation to airway diameter. We compared airway smooth muscle based both on the diameter of the airway and the actual position of each airway within the airway tree, unlike most human studies, which have been restricted to only using airway size and not position because of limited lung samples. The relationship that we found between airway size and the amount of smooth muscle (more smooth muscle in larger airways) has been previously reported for distal bronchioles in the lungs of humans with fatal asthma [8–10].

While an increased amount of airway smooth muscle (smooth muscle hypertrophy) is a well-recognized characteristic of remodelled airways in allergic asthma, how this increase in smooth muscle is accomplished is not clear. Smooth muscle is organized into bundles [34]. The increase that appears as more smooth muscle tissue around an airway

has been measured on histological sections as an increase in the proportion of the airway wall occupied by smooth muscle or in the volume of smooth muscle per unit area or length of the airway perimeter, as we found. This increase could be because of an increased number of bundles occupying the wall, which is not the case for the distal airways we evaluated (abundance did not increase) or an enlargement of individual bundles, which is the case for some of the airways we evaluated. Individual bundles could appear to be enlarged because of increases in myocyte size or increases in number of myocytes per bundle. Which of these is the case for our study is not clear.

The processes regulating the change in smooth muscle in the walls of airways undergoing rapid increases in size during postnatal lung growth are not understood. Our study suggests that much of the increase in smooth muscle mass associated with allergic asthma may be because of either a perturbation of the morphogenic events that regulate airway smooth muscle bundle size as airways grow or abnormal growth of the smooth muscle itself. An increase in smooth muscle is only one of a number of changes to airway wall components that can contribute to airway hyperactivity. Airway wall components appear to act as integral parts of the airway epithelial–mesenchymal trophic unit in maintaining airway homeostasis or in responding to allergic stress, producing airway remodelling [35–37]. In asthmatics with extensively remodelled airways and airway hyper-reactivity, the following cellular and acellular components of the wall are reorganized: epithelium, basement membrane zone, attenuated fibroblast sheet, smooth muscle, and resident and migrating inflammatory and immune cells. In the animals that were evaluated in this study, an increase in baseline airway resistance and a decrease in the amount of histamine necessary to produce a 150% increase in airway resistance at the end of the 5-month study period were found, but these differences were not statistically significant [20]. In addition, these animals exhibited many of the changes associated with allergic airway disease in humans. They had elevated mucus goblet cell numbers in mid-level airways, a three- to four-fold increase in eosinophils in both proximal and terminal airway mucosa, a thicker basement membrane zone, and a marked disarrangement of epithelial innervation to the mid-level and more distal conducting airways [20, 25, 38]. When oxidant stress, through exposure to ozone, is added to the HDMA allergen exposure there is an even greater airway hyper-reactivity and elevated baseline resistance [20]. Other changes in these airways were more marked, especially as they relate to the distribution of nerve fibers in the airway epithelial surface [38] and in the function of the nucleus tractus solitarius of the main brain stem [39]. Reactivity of airways to inhaled allergens and other stimulants appears to involve any number of factors including the afferent central and efferent limbs of the neural reflex pathways; the presence or redistribution of reactive cells (such as mast cells and eosinophils) that secrete smooth muscle stimulants; and alterations in the smooth muscle itself, which provide for increased force and more rapid response to allergen stimulation.

Our study in infant monkeys has shown that smooth muscle thickening, a component of airway remodelling, most likely is produced by an increase in the size of smooth muscle bundles. The normal developmental pattern appears to be maintaining

airway bundle size once the airway begins to grow. Control of smooth muscle proliferation is likely key to maintaining smooth muscle bundle density [40]. Previous studies suggest that soon after birth proliferation increases in smooth muscle bundles which then decreases with increasing age [41]. Many factors influence postnatal respiratory development. Perlecan, an extracellular matrix proteoglycan known to store and regulate growth factors, is an example of an airway wall component whose abundance is high at birth when proliferation is high and decreases postnatally as proliferation decreases [41]. Smooth muscle itself can be a source of regulatory factors with a potential autocrine role and many of them can be stimulated by the presence of immunomodulatory cytokines associated with asthma, such as IL-1 β and TNF- α [42]. How prominent a role each of these factors may play during development of smooth muscle, especially under conditions of allergen challenge is unclear, but our study establishes that postnatal allergen exposure does alter smooth muscle development to produce bundles larger than would be expected for the age of the animals and the size of the airways.

In summary, our study shows that postnatal exposure to allergen alters maturation of conducting airways by increasing smooth muscle mass and bundle size. Although the regulatory processes that drive smooth muscle morphogenesis in airways are poorly understood, our study suggests that there are critical windows when postnatal exposure to an allergen during these developmental events can disrupt their normal pattern and alter normal smooth muscle organization in favour of the increases in smooth muscle found in young human asthmatics.

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