

- decline in lung function than non-Hispanic whites. *Am J Respir Crit Care Med* 2011;184:1254–1260.
16. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, *et al.* Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011;43:887–892.
 17. Rosas-Salazar C, Apter AJ, Canino G, Celedón JC. Health literacy and asthma. *J Allergy Clin Immunol* 2012;129:935–942.
 18. Fritz GK, McQuaid EL, Kopel SJ, Seifer R, Klein RB, Mitchell DK, Esteban CA, Rodriguez-Santana J, Colon A, Alvarez M, *et al.* Ethnic differences in perception of lung function: a factor in pediatric asthma disparities? *Am J Respir Crit Care Med* 2010;182:12–18.
 19. Wu AC, Smith L, Bokhour B, Hohman KH, Lieu TA. Racial/ethnic variation in parent perceptions of asthma. *Ambul Pediatr* 2008;8:89–97.
 20. Fox P, Porter PG, Lob SH, Boer JH, Rocha DA, Adelson JW. Improving asthma-related health outcomes among low-income, multiethnic, school-aged children: results of a demonstration project that combined continuous quality improvement and community health worker strategies. *Pediatrics* 2007;120:e902–e911.
 21. Cloutier MM, Jones GA, Hinckson V, Wakefield DB. Effectiveness of an asthma management program in reducing disparities in care in urban children. *Ann Allergy Asthma Immunol* 2008;100:545–550.
 22. Bailey EJ, Cates CJ, Kruske SG, Morris PS, Brown N, Chang AB. Culture-specific programs for children and adults from minority groups who have asthma. *Cochrane Database Syst Rev* 2009;CD006580.

Copyright © 2012 by the American Thoracic Society
DOI: 10.1164/rccm.201202-0350ED

More Muscle in Asthma, but Where Did It Come From?

The airway wall of individuals with asthma displays prominent structural changes including the following: areas of epithelial loss, the presence of intraepithelial eosinophils, goblet cell metaplasia, subepithelial reticular basement membrane thickening, increased vascularity, and inflammatory cell infiltration of the subepithelial connective tissue. Thickened layers of airway smooth muscle (ASM) lie closer to the epithelium (1, 2). ASM hyperplasia is more prominent in severe asthma. Hypertrophy of ASM in proximal airways has been reported in biopsy (1) and in *post mortem* studies (3), but is not always observed (4). These structural changes continue to intrigue those active in asthma research, because they are linked to airway hyperresponsiveness, fixed airway obstruction, and loss of bronchodilator response to deep inspiration. However, the nature and extent of these functional impacts on asthma severity is still debated, as evidence is limited to inferences from correlative studies.

The article by James and colleagues in this issue of the *Journal* (pp. 1058–1064) comprises the largest systematic structural investigation of *post mortem* airways in asthma (5). The consolidation of data from multiple centers required careful analysis of potential systematic differences, as fixation, sampling, and specimen availability differed between some of the collaborating centers. The investigators used a stereological approach to focus on the volume occupied by ASM and the composition of this volume: the numerical density of ASM, the size of ASM, and the volume of extracellular matrix (ECM) within the ASM layer. Tissue was stained with Masson's trichrome on thin 0.5- μ m sections to estimate areas occupied by ECM or ASM cells, and hematoxylin was used for thick 30- μ m sections analyzed using an optical disector to estimate the numerical density of ASM cells. Although the presence of a thickened ASM layer in individuals with asthma is uncontroversial, the reasons for it are still contested, with hyperplasia, hypertrophy, and ECM expansion all thought to contribute. James and coworkers report that ASM hypertrophy in nonfatal and fatal asthma is evident in more proximal airways, whereas hyperplasia is detected in all airways of fatal asthma, but is limited to proximal airways of nonfatal asthma. The hypertrophic response is modest by comparison with the increase in ASM number. The volume of ECM in fatal asthma is greater than either the nonfatal asthma or the control groups, but the fractional area occupied by ECM declined significantly in fatal asthma. This decline is interesting in light of recent studies showing that ECM can be remodeled by ASM to markedly

reduced volumes and much tighter fibril packing by processes that are not inhibited by β_2 -adrenoceptor agonists agents or glucocorticoids (6, 7). Whether the ECM density (i.e., dehydrated weight/volume) is increased in fatal asthma remains to be established. Collectively, the findings of James and colleagues suggest that hyperplasia, rather than either hypertrophy or ECM expansion, is the dominant reason for the increase in ASM volume.

The principal conclusion of James and coworkers is that there is little effect of asthma duration on the extent of smooth muscle layer thickening (5). Analysis of asthma severity did not reveal further significant associations beyond those identified by comparison of fatal and nonfatal cases. The apparent lack of effect of asthma duration on the extent of airway remodeling is consistent with a number of studies suggesting that remodeling is an early event in the natural history of asthma. ASM hyperplasia has been reported in biopsies of children with asthma of short duration (8), as has reticular basement membrane thickening (9). When is the formative period for the ASM hyperplasia, and remodeling more generally? How dynamic are these processes throughout the course of asthma in different patient populations? Is severe pediatric asthma characterized, not only by different drivers of inflammation (10), but also distinct processes of remodeling? Indeed, the findings of James and colleagues are not inconsistent with the notion of “premodeling”: that the defect in smooth muscle may precede diagnosable asthma, and perhaps even precede the onset of airway inflammation (9). There is circumstantial evidence that ASM hyperplasia constitutes a response to inflammation. Airway smooth muscle in culture is stimulated to proliferate by a diverse set of inflammatory mediators present in asthma. Moreover, induction of inflammation in mice, rats, guinea pigs, and nonhuman primates is sufficient to induce airway smooth muscle hyperplasia and many of the other features of airway wall remodeling (11). Based on these findings, we would expect to observe evidence of dynamic remodeling in inflamed airways. Work in animal models of inflammation-induced airway remodeling demonstrates an increase in the fraction of ASM within the airway showing markers of cell cycle progression (i.e., proliferation). However, several studies have reported that ASM cells of individuals with severe asthma do not show increased frequency of expression of cell cycle progression markers, such as cyclin D1 and proliferating cell nuclear antigen (PCNA) in *post mortem* specimens (12), or in biopsies, measuring either cyclin D1 (2) or Ki67 (13). A more recent biopsy study reports increases in Ki67 and proliferating cell nuclear antigen frequency in severe asthma (14). Although there is clearly a need for further studies

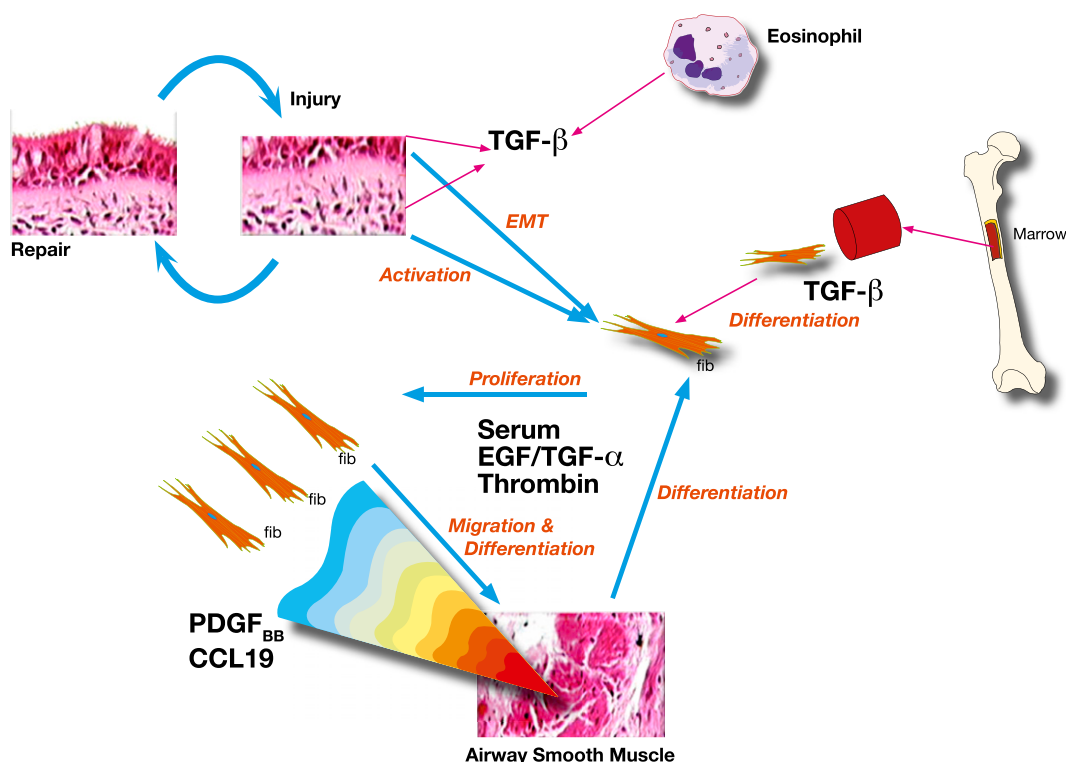


Figure 1. The origins of excess airway smooth muscle (ASM) in asthma are uncertain, but the process is frequently viewed as an injury/repair cycle that is persistent, because resolution is not achieved. The most obvious explanation, that ASM within the ASM bundles undergoes proliferation, is not well evidenced in human airways. Alternative suggestions include the recruitment of activated (myo)fibroblasts from the subepithelial region to the ASM bundle. The fibroblast population of this region may be supplemented by fibrocytes recruited from the bone marrow through the circulation to the inflamed airway (19). On the other hand, ASM can adopt a motile phenotype, enabling its migration from the antiproliferative fibrillar collagen and proteoglycan-rich extracellular matrix of the ASM bundles to the inflamed subepithelial region where ex-

tracellular matrix breakdown products comprise a microenvironment conducive to proliferation. Recent work raises the possibility that TGF- β may induce EMT in the airway epithelium (20). All these alternative explanations of ASM hyperplasia require cycles of differentiation and migration. There is good evidence for expression of active TGF- β in sufficient concentrations to differentiate fibroblasts or epithelium, and to induce hypertrophy of ASM. In addition, serum from leaky bronchial blood vessels, PDGFBB, and the chemokine CCL19 are all known to induce ASM migration. CCL = chemokine ligand; EGF = epidermal growth factor; EMT = epithelial-mesenchymal cell transition; fib = fibroblast; PDGFBB = platelet-derived growth factor BB; TGF = transforming growth factor.

to address the extent to which remodeling is stable or dynamic, the apparent stability of the ASM population and the relative lack of impact of asthma duration on ASM thickening add further weight to the case that the ASM hyperplasia is an early-life event. Although β_2 -adrenoceptor agonists and glucocorticoids attenuate smooth muscle proliferation under some conditions *in vitro* (15), if the active phase of remodeling is early in life, possibly before asthma is diagnosed, then treatment may not be commenced in time to prevent the ASM component of the remodeling. If such changes are not dynamic in established asthma, then later treatment may also have little impact.

If ASM hyperplasia is established early in the course of asthma, or even before its diagnosis, then approaches to therapeutic targeting of ASM need to reflect different opportunities and a better understanding of the origins of hyperplastic ASM (Figure 1). Recent models of functional consequences of airway remodeling, incorporating a more sophisticated appreciation of airway geometry and biomechanics, predict, like earlier models, that more ASM hyperplasia would lead to airway hyperresponsiveness (16). However, the extent of the impact is dependent on the relative abundance of contractile and synthetic phenotype of ASM cells comprising the hyperplastic ASM. There is emerging evidence of regional heterogeneity in ASM cell expression of markers of ASM phenotype, such as the regulator of G-protein signaling (RGS4), which is elevated in severe asthma, expressed in cells at the periphery of ASM bundles, and marks a noncontractile, proliferative ASM phenotype (17). There is also increasing interest in cytoskeletal elements within the ASM cells that contribute to cell stiffness (18) independently of acto-myosin filament-mediated smooth muscle shortening. The phenotypic/cytoskeletal

changes in ASM may provide alternative and more tractable opportunities for drug targeting than does ASM hyperplasia.

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

ALASTAIR STEWART, PH.D.
Department of Pharmacology
University of Melbourne
Parkville, Victoria, Australia

References

1. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated to severe asthma. *Am J Respir Crit Care Med* 2003;167:276–282.
2. Ward JE, Harris T, Bamford T, Mast A, Pain MC, Robertson C, Smallwood D, Tran T, Wilson J, Stewart AG. Proliferation is not increased in airway myofibroblasts isolated from asthmatics. *Eur Respir J* 2008;32:362–371.
3. Ebina M, Yaegashi H, Chiba R, Takahashi T, Motomiya M, Tanemura M. Hyperreactive site in the airway tree of asthmatic patients revealed by thickening of bronchial muscles: a morphometric study. *Am Rev Respir Dis* 1990;141:1327–1332.
4. Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, Solberg OD, Carter R, Wong HH, Cadbury PS, Fahy JV. Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 2004;169:1001–1006.
5. James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, Bai TR, Abramson MJ, McKay KO, Green FH. Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am J Respir Crit Care Med* 2012;185:1058–1064.
6. Bourke JE, Li X, Foster SR, Wee E, Dagher H, Ziogas J, Harris T, Bonacci JV, Stewart AG. Collagen remodelling by airway smooth

- muscle is resistant to steroids and beta-agonists. *Eur Respir J* 2011;37:173–182.
7. Schuliga M, Ong SC, Soon L, Zal F, Harris T, Stewart AG. Airway smooth muscle remodels pericellular collagen fibrils: implications for proliferation. *Am J Physiol Lung Cell Mol Physiol* 2010;298:L584–L592.
 8. Regamey N, Ochs M, Hilliard TN, Muhlfeld C, Cornish N, Fleming L, Saglani S, Alton EW, Bush A, Jeffery PK, *et al.* Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2008;177:837–843.
 9. Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. *Thorax* 2005;60:389–394.
 10. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, Tsartsali L, Lloyd CM, Bush A, Saglani S. Pediatric severe asthma is characterized by eosinophilia and remodeling without t(h)2 cytokines. *J Allergy Clin Immunol* 2012;129:974–982.
 11. Meurs H, Gosens R, Zaagsma J. Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models. *Eur Respir J* 2008;32:487–502.
 12. James A, Carroll M, Dromey J, Down K, Elliot J, Mutavdzic S, Carroll N. In-situ proliferation of inflammatory cells and smooth muscle cells in patients with and without asthma. *Respirology* 2002;7:A11.
 13. Benayoun L, Druihe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;167:1360–1368.
 14. Hassan M, Jo T, Risse PA, Tolloczko B, Lemiere C, Olivenstein R, Hamid Q, Martin JG. Airway smooth muscle remodeling is a dynamic process in severe long-standing asthma. *J Allergy Clin Immunol* 2010;125:1037–1045.e3.
 15. Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, Herszberg B, Lavoie JP, McVicker CG, Moir LM, *et al.* Proliferative aspects of airway smooth muscle. *J Allergy Clin Immunol* 2004;114:S2–S17.
 16. Brook BS, Peel SE, Hall IP, Politi AZ, Sneyd J, Bai Y, Sanderson MJ, Jensen OE. A biomechanical model of agonist-initiated contraction in the asthmatic airway. *Respir Physiol Neurobiol* 2010;170:44–58.
 17. Damera G, Druey KM, Cooper PR, Krymskaya VP, Soberman RJ, Amrani Y, Hoshi T, Brightling CE, Panettieri RA Jr. An rgs4-mediated phenotypic switch of bronchial smooth muscle cells promotes fixed airway obstruction in asthma. *PLoS ONE* 2012;7:e28504.
 18. Zhang W, Gunst SJ. Interactions of airway smooth muscle cells with their tissue matrix: implications for contraction. *Proc Am Thorac Soc* 2008;5:32–39.
 19. Saunders R, Siddiqui S, Kaur D, Doe C, Sutcliffe A, Hollins F, Bradding P, Wardlaw A, Brightling CE. Fibrocyte localization to the airway smooth muscle is a feature of asthma. *J Allergy Clin Immunol* 2009;123:376–384.
 20. Hackett TL, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky DV, Murray LA, Argentieri R, Kicic A, Stick SM, Bai TR, *et al.* Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. *Am J Respir Crit Care Med* 2009;180:122–133.

Copyright © 2012 by the American Thoracic Society
DOI: 10.1164/rccm.201203-0457ED

The Microbiota in Respiratory Disease

Evidence derived from culture-independent approaches has revealed that the lungs, which were previously considered a sterile microenvironment during health, contain microbial populations. Furthermore, using these culture-independent methodologies, we are now beginning to appreciate that diseased lungs can harbor microbial communities that may contribute to disease pathogenesis and progression. The study by Sze and colleagues (1) in this issue of the *Journal* (pp. 1073–1080) follows those by Hilty and colleagues (2), Erb-Downward and colleagues (3), Huang and colleagues (4), and Charlson and colleagues (5) and continues to expand our nascent understanding of the composition of the pulmonary microbiome. Remarkably, despite enormous differences in technical execution and data analysis methodology, the results of the study by Sze and coworkers are generally consistent with previously published reports (2–5). These studies utilized bronchial brushings, bronchoalveolar lavage specimens, endotracheal aspirates, and lung tissue derived aseptically from lung explants, whereas Sze and colleagues are the first to report analyzing the microbiome in lung tissue biopsies. Of particular interest is that, despite technical differences, the study by Sze and coworkers is consistent with the studies by Erb-Downward and colleagues in concluding that Global Initiative for Chronic Obstructive Lung Disease 4 chronic obstructive pulmonary disease (COPD) lungs have a different microbiome than nonsmokers or healthy smokers. Sze and colleagues also directly compare the COPD lung to the cystic fibrosis lung to demonstrate microbiome differences between these lung diseases. These findings are also consistent with the previously published individual culture-independent studies of the cystic fibrosis lung microbiome and COPD microbiome, not to mention consistent with older culture-based studies that highlight differences in the microbiology of these two respiratory diseases.

The most significant differences between the current and previous studies are in the technical and computational approaches used. Due to the low target DNA content of their samples, the investigators deviated from the methods accepted by the Human

Microbiome Project (HMP) (http://www.hmpdacc.org/doc/HMP_MDG_454_16S_Protocol_V4_2_102109.pdf) and utilized a nested polymerase chain reaction approach with extremely high cycle numbers. The HMP avoided the use of nested polymerase chain reaction in its analyses because, under the conditions required for this approach, even a sterile water control can appear to have a significant bacterial community. Sze and colleagues present a control in their analysis (given the cycling conditions, the simple subtraction of a low background, as is used in most studies, would not suffice), so that it can clearly be seen that the artifact “community” of the water is different from the bacterial community of the lung tissue biopsies. From the raw sequences, the authors analyze the data partially by using a well-established method for 16S data analysis (for the purposes of sequence classification) (6) and by also using a much less vetted method for clustering of the sequences into operational taxonomic units (7). Exactly how these differences in methodology will affect 16S data analysis remains unclear. It would be interesting and important to see a head-to-head comparison of these operational taxonomic unit calling methods in future publications.

However, despite the many reasons that there should be differences between this current study and those that have come before, it is important to note that there is broad agreement in results, i.e., diseased lungs harbor microbial communities that differ from those of healthy individuals. The impact of these microbial differences is still far from understood, but the potential for providing novel insights into the pathophysiology of respiratory disease is promising.

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

JOHN R. ERB-DOWNWARD, Ph.D.

GARY B. HUFFNAGLE, Ph.D.

FERNANDO J. MARTINEZ, M.D., M.S.

*Division of Pulmonary and Critical Care Medicine
University of Michigan Health System
Ann Arbor, Michigan*

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting <http://www.adobe.com/products/acrobat/readstep2.html>.

For more assistance with Adobe Reader visit <http://www.adobe.com/support/products/acrreader.html>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.