Pulmonary Perspective

Airway Smooth Muscle in Bronchial Tone, Inflammation, and Remodeling

Basic Knowledge to Clinical Relevance

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Airway smooth muscle (ASM) plays a pivotal role in modulating bronchomotor tone but also orchestrates and perpetuates airway inflammation and remodeling. Despite substantial research, there remain important unanswered questions. In 2006, the National Heart, Lung, and Blood Institute sponsored a workshop to define new directions in ASM biology. Important questions concerning the key functions of ASM include the following: Does developmental dysregulation of ASM function promote airway disease, what key signaling pathways in ASM evoke airway hyperresponsiveness *in vivo*, do alterations in ASM mass affect excitation-contraction coupling, and can ASM modulate airway inflammation and remodeling in a physiologically relevant manner? This workshop identified critical issues in ASM biology to delineate areas for scientific investigation in the identification of new therapeutic and diagnostic approaches in asthma, chronic obstructive pulmonary disease, and cystic fibrosis.

Keywords: myocyte; signal transduction; force generation; migration; remodeling

Diseases characterized by airway obstruction—namely, asthma, chronic bronchitis, emphysema, and cystic fibrosis—induce substantial morbidity and mortality in the United States. Although the precise mechanisms promoting airway obstruction in these common diseases remain unclear, airway smooth muscle (ASM) shortening likely plays a significant role in disease pathogenesis (1–3). Indeed, a mainstay in the treatment of airway obstruction is the use of bronchodilators, whose primary function is to relax ASM. Substantial progress in the past 20 years in ASM biology has identified ASM not only as modulating bronchomotor tone but as orchestrating and perpetuating inflammation and fibrosis in airway remodeling. As shown in Figure 1, ASM serves a variety of functions that are interrelated; however, in disease, bronchial

smooth muscle may serve a distinct role in the pathogenesis. Despite existing controversy over the role of ASM function in health (4), few argue that, in the pathogenesis of diseases of airway obstruction, smooth muscle plays a pivotal role (5–7). Given the recent focus of ASM as a potential immunomodulatory cell as well as a cell important in airway remodeling, there remain substantial gaps in our understanding of ASM function. Furthermore, ASM cells manifest diverse phenotypes that induce functional plasticity concerning force generation, growth, and migration (8).

New therapeutic approaches, such as bronchial thermoplasty, in part eliminate bronchial ASM (9). Although this therapy remains investigational, such approaches targeting the elimination of ASM will likely serve as useful experimental tools to address the relative contribution of ASM in modulating airway inflammation and remodeling.

The goal of this perspective is not to provide an exhaustive compendium of studies in ASM biology in health and disease but to provide a focus for future studies that will enhance our understanding and provide new therapeutic targets in modulating myocyte function. The National Heart, Lung, and Blood Institute (NHLBI) convened a workshop on September 11 and 12, 2006, to better understand the complex role of ASM in health and disease and to identify strategies and future studies and to identify important gaps in our knowledge. (A list of participants in the workshop may be found before the REFERENCES.) The topics addressed by the participants were wide ranging; however, all focused on ASM biology. The major theme of this symposium was to address the developmental biology of ASM; excitation-contraction coupling; myocyte hypertrophy, hyperplasia, and migration; as well as the role of ASM as an immunomodulatory cell and a cell that promotes airway remodeling and potentially angiogenesis through matrix secretion. The discussion surrounding the above issues identified important research areas and questions that were formulated from clinicians and experts in the fields of inflammation, immunology, pharmacology, biophysics, cell biology, and physiology. This article summarizes these discussions and attempts to identify key issues that should be the focus of future research in ASM biology.

DEVELOPMENTAL BIOLOGY

Evidence demonstrates that from embryogenesis onward ASM elaborates smooth muscle-specific actin and is mechanically active (10, 11). Despite controversy as to whether the ASM plays a significant role in health, studies suggest that prenatal airway peristalsis is developmentally regulated during lung

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Figure 1. Airway smooth muscle (ASM) function in health and disease. ASM, believed to be the pivotal cell in modulating bronchomotor tone, also undergoes phenotypic alteration manifested by proliferation, hypertrophy, and migration, as well as in the expression of chemokines and cytokines important in promoting or inhibiting airway inflammation.

growth in a manner that appears dependent on specific growth factors and epithelial–mesenchymal interactions. Conceivably, ASM may also modulate angiogenesis and vasculogenesis via secretion of paracrine molecules (12). Despite recent progress in the area of ASM developmental biology, there remain substantial gaps in our understanding. Important unanswered questions include the following:

- What local factors regulate ASM development in embryogenesis?
- Are the intracellular signaling pathways important in the development of ASM also manifested in the pathology of ASM in the adult animal?
- What developmentally regulated patterns of protein expression in ASM are recapitulated in the adult in health and in asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis?
- Do therapeutic approaches that eliminate ASM *in utero* abrogate airway disease in the adult?
- Are there distinct progenitor cells or precursors that can be identified in developing ASM that exist in the adult?

EXCITATION–CONTRACTION COUPLING

Over the past 20 years, progress has occurred in understanding ion channels, receptors, and the biophysics of excitation–contraction coupling in ASM at the cellular and molecular levels (2, 13–17). Despite such progress, the mechanisms that underlie spontaneous tone in inflammatory bronchoconstriction and alterations in the phenotype of smooth muscle cells that accompany airway inflammation remain unknown. In asthma, evidence suggests that intrinsic alterations in ASM excitation–contraction coupling exist as compared with tissue from healthy subjects (18–22). Greater focus on the role of intracellular calcium release channels, the importance of the mechanisms of calcium sensitization, and changes of expression of ion channels, receptors, and second messengers is needed in physiologically relevant preparations and in human tissue. Extensive remodeling occurs in the airways of some patients with asthma and chronic obstructive lung disease; however, neither the signals eliciting these alterations in phenotype nor the resulting changes in gene expression relevant to excitation–contraction coupling are understood. In addition, mechanical adaptations manifested by alterations in contractile and cytoskeletal protein expression may promote airway hyperresponsiveness in asthma, COPD, and cystic fibrosis. New models and genetic tools that characterize these processes in physiologically relevant contexts would bridge a critical gap in our current understanding. Important research questions include the following:

- What is the role of spontaneous calcium release events in the generation of airway tone?
- What are the regulatory molecules that control intracellular calcium channel function?
- What are the spontaneous release events regulating tone in small airways?
- What are the differences in ion channel expression between small and large airways?
- What are the molecular mechanisms of depolarizing chloride current in ASM cells?
- Do adaptive phenotypes alter the expression and function of sarcolemmal ion channels? Are these phenotypes distinct in asthma, COPD, and cystic fibrosis?
- What is the role of calcium sensitization in the control of small airway tone?
- What modifications of contractile and cytoskeletal proteins induce dynamic remodeling and render the ASM hyperresponsive?
- What are the stimuli that elicit mechanical adaptation and the proteins necessary for adaptation that modulate the physical nature of ASM cells?

CELL PROLIFERATION, GROWTH, AND MIGRATION

Early studies in the pathogenesis of asthma and COPD demonstrate that ASM mass is altered (23–25). In some diseases, ASM mass increases due to coordinated increase in size (hypertrophy) and number (hyperplasia) of myocytes (25, 26). Furthermore, after airway injury, there appears to be myocyte migration that could serve an important regulatory role in the generation of ASM mass and in airway repair (6, 27). Despite remarkable progress in understanding the signal transduction pathways that modulate ASM cell proliferation, the ultimate role of ASM mass in regulating bronchomotor tone or in the manifestation of irreversible airflow obstruction remains obscure (28, 29). Importantly, recent evidence in cultured ASM cells from subjects with asthma suggests a unique phenotype in comparison to cells derived from healthy subjects (30–32). Given the changes in smooth muscle mass in disease, therapeutic approaches aimed at decreasing ASM mass or preventing myocyte migration may offer new therapeutic targets in asthma and COPD. Important future questions include the following:

- What is the physiologic relevance of increasing ASM mass?
- Do increases in ASM mass occur throughout the tracheo-bronchial tree or are they restricted to certain segments of the airway?
- What are the cytokines and growth factors that primarily modulate ASM mass?
- What are the critical translational and transcriptional control pathways regulating ASM hypertrophy?
- Are the processes seen in developmental biology of ASM recapitulated distinctly in asthma, COPD, or cystic fibrosis?
- Concerning myocyte migration, what are the pools of cells responsible for the migration of mesenchymally derived cells into the airway?
- Do ASM cells migrate or are there potentially intravascular pools or hemopoietically derived pools of progenitors that home to ASM?
- What is the physiologic relevance of ASM migration?

IMMUNOBIOLOGY

Although excitation–contraction coupling of ASM may play an important role in bronchomotor tone, ASM can function as an immunomodulatory cell (33, 34). Primarily on the basis of *in vitro* studies, ASM has been found to secrete a variety of chemokines and cytokines that can orchestrate and perpetuate the function of trafficking leukocytes, airway inflammation, and angiogenesis (as shown in Figure 2). ASM may also serve to extinguish airway inflammation via eicosanoid and prostanoid secretion. Few studies have focused on ASM secretion of chemokines and cytokines *in vivo*, and the relative contribution of this cell type in the modulation of airway inflammation in disease remains unclear. In addition, ASM can directly bind trafficking leukocytes and other resident cells and can modulate the function of such cells (35–39). Important questions to be addressed include the following:

- *In vivo*, are chemokines, cytokines, and growth factors expressed by ASM?
- What is the physiologic relevance of ASM-derived chemokines and cytokines? Can these mediators also modulate angiogenesis?
- Can therapeutic approaches be developed to inhibit ASM-derived immunomodulatory molecules or stimulate ASM-derived antiinflammatory mediators, and what are the consequences of such therapeutic approaches?
- Does ASM modulate epithelial cell, lymphocyte, and mast cell function, and secretion of pro- or antiinflammatory mediators *in vivo*?
- Is ASM a target for antiinflammatory mediators and what is the role of glucocorticoids in modulating ASM function *in vivo*?

- Can ASM serve as a model or platform to evaluate novel antiinflammatory agents?

MATRIX

ASM is surrounded by a rich matrix scaffolding. ASM also serves as an important source of matrix expression and deposition (40, 41). Both *in vivo* and in cultured human ASM, there appears to be a resistance to apoptosis, suggesting a strong survival signal likely mediated in part through cell adhesion–matrix interactions (42–44). Furthermore, extracellular matrix through binding of cell surface molecules modulates intracellular signaling and affects excitation–contraction coupling, proliferation of ASM, and β_2 -adrenergic receptor–mediated relaxation (39, 40). The following important unanswered questions remain:

- Can matrix expression define the major mesenchymal cell lineages in the airways that modulate ASM phenotype?
- Which cells are the major controllers of matrix deposition in the airway *in vivo* in virus- and/or allergen-induced airway hyperresponsiveness models?
- What are the relative contributions of matrix deposition to airway remodeling, and the development of fixed airway obstruction in asthma, COPD, or cystic fibrosis?
- Are increases in ASM mass due to alterations in matrix secretion as well as in alterations of myocyte number and size?
- What are the determinants of matrix deposition and turnover in the airway wall?

Figure 2. A proposed role of airway smooth muscle (ASM) function in modulating airway inflammation. ASM appears to be an important end organ whose function is altered by mediators secreted by epithelium and leukocytes; however, new evidence suggests that ASM itself may orchestrate and regulate the function of other structural cells as well as trafficking leukocytes that ultimately impact on airway inflammation and bronchoconstriction. CAM = cell adhesion molecules.

TABLE 1. GENERAL RECOMMENDATIONS OF THE WORKING GROUP

1. Establish collaborative networks to obtain a repository of bronchial tissue to assess ASM function in health and disease.
2. Develop important biomarkers based on proteomic and genomic approaches that can predict ASM function.
3. Investigate new methods that link *in vitro* observations to *in vivo* disease state with regard to ASM function.
4. Identify potential stem cells and progenitors of ASM and signaling pathways important in the phenotype switching of myocytes in disease.
5. Develop relevant animal models that target ASM expression to define the role of this cell in immunomodulation and in the development of airway remodeling in irreversible airflow obstruction.
6. Develop novel approaches for targeting the elimination of ASM.

Definition of abbreviation: ASM = airway smooth muscle.

CONCLUSIONS

Extraordinary progress has occurred in the area of ASM biology. The recognition of ASM as more than a contractile unit critical in the regulation of bronchomotor tone has opened new vistas to our understanding of airway biology. Evidence suggesting that targeted elimination of ASM improves outcomes in asthma is exciting (9). Despite considerable research in the areas of excitation–contraction coupling, growth, migration, immunobiology, and matrix expression by ASM, there remain substantial gaps in our understanding of how these processes occur *in vitro* and relate to the *in vivo* disease state. Contributors to this symposium stated that a large unmet need exists in the linking of the *in vitro* ASM studies to the *in vivo* state and suggested a coordinated repository for tissues obtained from patients with well-characterized phenotypes to assess the role of ASM function in the pathogenesis of disease. A summary of other important recommendations is described in Table 1. By far, ASM plays a critical role in airways obstruction in a variety of important diseases that impact on morbidity and mortality in the United States. ASM remains a critical therapeutic target, and filling the gaps in our understanding of the role of ASM in the pathogenesis of disease will have a profound impact in patients with asthma, COPD, and cystic fibrosis.

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