

Airway smooth muscle pathophysiology in asthma



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1. To describe new concepts about airway smooth muscle characteristics in health and asthma.
2. To recognize the beneficial effect of deep breathing in normal respiratory function.
3. To understand the complexity of asthma genetics and epigenetics.
4. To review emerging therapeutic strategies which could lead to airway smooth muscle relaxation in asthma.

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The airway smooth muscle (ASM) cell plays a central role in the pathogenesis of asthma and constitutes an important target for treatment. These cells control muscle tone and thus regulate the opening of the airway lumen and air passage. Evidence indicates that ASM cells participate in the airway hyperresponsiveness as well as the inflammatory and remodeling processes observed in asthmatic subjects. Therapeutic approaches require a comprehensive understanding of the structure and function of the ASM in both the normal and disease states. This review updates current knowledge about ASM and its effects on airway

narrowing, remodeling, and inflammation in asthma. (*J Allergy Clin Immunol* 2021;147:1983-95.)

Key words: Airway smooth muscle, airway remodeling, asthma, deep inspiration, inflammation, bronchodilation, bronchoconstriction, bronchial thermoplasty, genetics, epigenetics, bitter taste receptors, olfactory receptors

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NEW CONCEPTS ON AIRWAY SMOOTH MUSCLE IN HEALTH

Airway smooth muscle structure

The airway smooth muscle (ASM) is composed predominantly of smooth muscle cells that mostly align along the axis of muscle shortening, and a network of intercellular connective tissue. Although the role of the ASM in healthy postnatal lungs remains controversial,^{1,2} a more complex picture of their function beyond just the regulation of bronchomotor tone is emerging. This includes secretory and immunomodulatory capabilities³ and being part of a postulated alarm system that senses exposure to allergens

Abbreviations used

AC:	Adenyl cyclase
AHR:	Airway hyperresponsiveness
AR:	Adrenergic receptor
ASM:	Airway smooth muscle
BK _{Ca} :	Large conductance Ca ²⁺ -activated K ⁺
BT:	Bronchial thermoplasty
[Ca ²⁺] _i :	Intracellular calcium concentration
cAMP:	Cyclic adenosine monophosphate
CHRM3:	M3 muscarinic receptor
CNG:	Cyclic nucleotide-gated channels
COX:	Cyclooxygenase
CPAP:	Continuous positive airway pressure
DI:	Deep inspiration
ECM:	Extracellular matrix
GABA:	γ-aminobutyric acid
GABA _A R:	GABA _A receptor
GPCR:	G protein-coupled receptor
GWAS:	Genome-wide association study
MCh:	Methacholine
miR:	MicroRNA
MLCK:	Myosin light chain kinase
MLCP:	Myosin light chain phosphatase
MYPT1:	Myosin phosphatase targeting subunit 1
NMDAR:	N-methyl-D-aspartate receptor
PG:	Prostaglandin
PKA:	Protein kinase A
ROCK:	Rho kinase
T2R:	Bitter taste receptor
TLR:	Toll-like receptor

and toxins and responds by activation of a chemosensory function⁴ that provokes life-preserving actions.

Unlike the crystal-like structure of the sarcomere in striated muscles, the microstructure of smooth muscles, as the name suggests, is disordered and amorphous,⁵ with evidence that its cytoskeleton is in a continuous state of remodeling.^{6,7} Moreover, the mechanical criteria and generic features of the ASM cell allow it to be classified as a soft glassy matter,⁸ a class that comprises foams, pastes, colloids, emulsions, and slurries. The soft glass theory of the ASM cell provides a framework to explain some of its puzzling mechanical aspects, including its uncharacteristic relaxation times that cannot be associated solely with myosin cross-bridge dynamics, its remarkable ability to both adapt its force-length relationship (called length adaptation) and remodel the cytoskeleton to become more fluid-like under stretch.⁹

ASM function

Muscle contraction is typically viewed as a mechanism that creates motion both in skeletal and in cardiac muscles or that establishes tone or maintains shape in smooth muscle of hollow organs. Because of the activity of breathing, the ASM is subject to a constant stress and strain load, which fluctuates. Tidal breathing induces muscle lengthening, which can effectively depress the active force generated.¹⁰ This endogenous bronchodilating response due to periodic stretch from breathing¹¹ or from involuntary (sigh) or voluntary deep inspirations (DIs)¹² prevents airway closure in normal individuals but is ineffective in asthma¹³ (Fig 1). Therefore, in the absence of this relaxing mechanism, the ASM is capable of exhibiting its full contractile potential, and

moderate ASM activation within an altered microenvironment could promote bronchospasm and acute exaggerated airway narrowing.¹⁴ One hypothesis to explain this impaired relaxing function suggests that the asthmatic ASM fails to achieve a melting, more fluid-like state of the contractile domain and benefit from the plasticity of the response, a proposed feature of smooth muscle to disassemble and reassemble its contractile apparatus to generate maximum force at different lengths.^{15,16}

The normal ASM exhibits other surprising characteristics in addition to the salutary effect of breathing and DI that antagonize bronchoconstriction. For example, ASM contains about one-fifth the myosin of skeletal muscle, yet the level of maximum active isometric force developed by the trachealis smooth muscle is equivalent to that of skeletal muscle. This could be attributed to the fact that the myosin filament in smooth muscle is longer than in skeletal muscle and thus could form more cross-bridges per sarcomere, resulting in more force. Another explanation is that the ASM shortens several times slower than the skeletal muscle,¹⁷ allowing longer contacting times between the actin and myosin to generate force. The enhanced shortening of the ASM of those with asthma versus those without asthma seen *ex vivo* could be attributed to exposure to elevated levels of procontractile agonists in asthmatic airways and/or to an intrinsic alteration of ASM contractility.¹⁸ *Ex vivo*, 90% of ASM shortening occurs in the first 3 seconds of a total 10-second duration of contraction. However, the shortening time of sensitized ASM is increased and contraction is completed entirely within 3 seconds.

Together, a better understanding of the biophysical properties of ASM demonstrates that muscle dynamics and plasticity as well as the interaction of the muscle with its load and environment are critical drivers in the ability of the ASM to narrow the airway.¹⁶

The human fetal ASM

Contrary to its controversial postnatal function, the role of the ASM in the fetus during pregnancy is essential for normal lung organogenesis.¹⁹ Bronchial myogenesis begins in the mesenchyme cells around the epithelial buds, which undergo sustained stretch and differentiate to express smooth muscle-specific proteins, for example, smooth muscle α-actin, smooth muscle myosin heavy chain, SM22, desmin, and calponin. The transcription factor serum response factor is crucial for the induction¹⁹ and maintenance²⁰ of smooth muscle-specific gene expression. Spontaneous contraction and relaxation of fetal human ASM present in the trachea and the primary and lobar bronchi at sixth to eighth week of life promote lung branching by facilitating fluid motion and changes in the intraluminal pressure. These provide mechanical cues for healthy growth of the airway tree.²¹ With a spiral orientation, ASM bundles wrap the fetal airway and become innervated and responsive to contractile and relaxing stimuli.^{22,23}

Following birth, the ASM transitions from a phasic contractile to a predominant tonic phenotype.²⁴ The presence of gap junctions and residual spontaneous activity described in mature airways are postulated as a mechanistic framework for the emergence of phasic ASM contraction, the frequency of which correlates with asthma severity²⁵ and could also contribute to airflow limitation and enhanced response to agonists.

The airway of healthy infants versus adults is more responsive, with greater maximal airway narrowing and sensitivity to induced bronchoconstriction (Fig 1). Likewise, fetal ASM is less sensitive to the bronchodilating and bronchoprotective effects of DI during

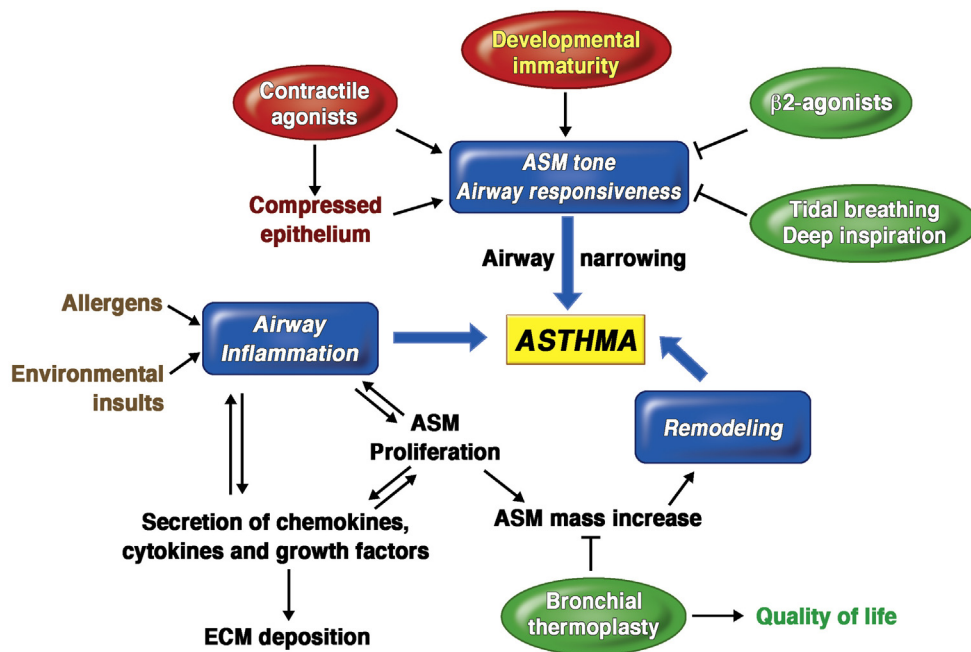


FIG 1. Role of ASM in asthma. ASM tone and airway responsiveness are increased after stimulation with contractile agonists, in prenatal ASM, and could result from the effects of a compressive stress on the airway epithelium. Normal breathing, deep inspiration, and administration of β_2 agonists decrease ASM responsiveness, opposing airway narrowing. In susceptible subjects, airway inflammation after exposure to allergens or other environmental insults leads to the proliferative/secretory ASM phenotype, which in turn contributes to the inflammatory process, the production of ECM, and overabundance of ASM, features of airway remodeling. BT ablates ASM, alleviates remodeling, and improves quality of life.

methacholine (MCh) challenge, a phenomenon similar to that in asthmatic adults.²⁶ A decline in responsiveness is thus observed from childhood to adolescence and parallels the increase in lung function. This improvement in respiratory function is less pronounced in females versus males.²⁷

Of interest, human fetal ASM cells exposed *ex vivo* to clinically relevant moderate hyperoxia used to treat premature babies exhibit a senescence phenotype of hypersecretion of proinflammatory and profibrotic mediators that, in a paracrine manner, promote contractile responses.²⁸ This finding indicates that the noxious effect of supplemental oxygen on prenatal ASM cells may contribute to the development of neonatal and pediatric asthma.

ASM IN ASTHMA

The ASM is a modulator of airways responsiveness. A salient feature of asthma is the loss of the homeostatic control of ASM. Any factor that decreases stress on the airways, including thickening of the lamina reticulosa or the peribronchial adventitia, shallow breathing, failure to take deep breaths, and inflammation, diminishes the beneficial effect of muscle load fluctuation and drives the airways to closure. These features are present in asthma.

Muscle contractility is the manifestation of the instantaneous relationship of force, velocity, length, and time²⁹; it captures all functions the muscle exerts including the capability to shorten and narrow the airway and to stiffen, bearing steady-state loads, coupled to ATP energy utilization. The interaction of the thick filament of myosin with the thin filament of actin and its

regulation are central to the contractile event; regulatory, accessory, scaffolding, and focal adhesion molecules also play a role in this process.

Numerous agents, including acetylcholine, histamine, endothelin-1, and bradykinin, activate ASM contraction. The canonical cholinergic pathway (Fig 2) involves activation of the M3 muscarinic receptor (CHRM3) after binding acetylcholine. CHRM3 couples with $G_{q,11}$ protein, which activates phospholipase C, an enzyme that catalyzes the formation of inositol triphosphate, promoting Ca^{2+} release from the sarcoplasmic reticulum. This increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which occurs as waves or Ca^{2+} oscillations, is a key step initiating and mediating ASM cell contraction.³⁰ Ca^{2+} binds calmodulin, and this complex activates myosin light chain kinase (MLCK). MLCK phosphorylates the serine-19 residue of the smooth muscle regulatory MLC20 subunit to induce a conformational change in myosin and causes actomyosin crossbridge formation and contraction (Fig 2). Muscle relaxation occurs when the myosin phosphatase PP1 subunit bound to MYPT1 (myosin phosphatase targeting subunit 1), within the myosin light chain phosphatase (MLCP) complex, removes the phosphate group from serine-19 of MLC20, reverting myosin to its original conformation unable to interact with actin. MYPT1 confers specificity for MLC20.³¹ Diacylglycerol, a product of phospholipase C catalytic function, activates protein kinase C, which phosphorylates CPI-17 to inhibit MLCP and favor contraction (Fig 2). Thus, force is generated by activation of MLCK and/or inhibition of MLCP and, conversely, a decrease in MLCK or increase in MLCP functions results in dilatation of the ASM (Fig 2).

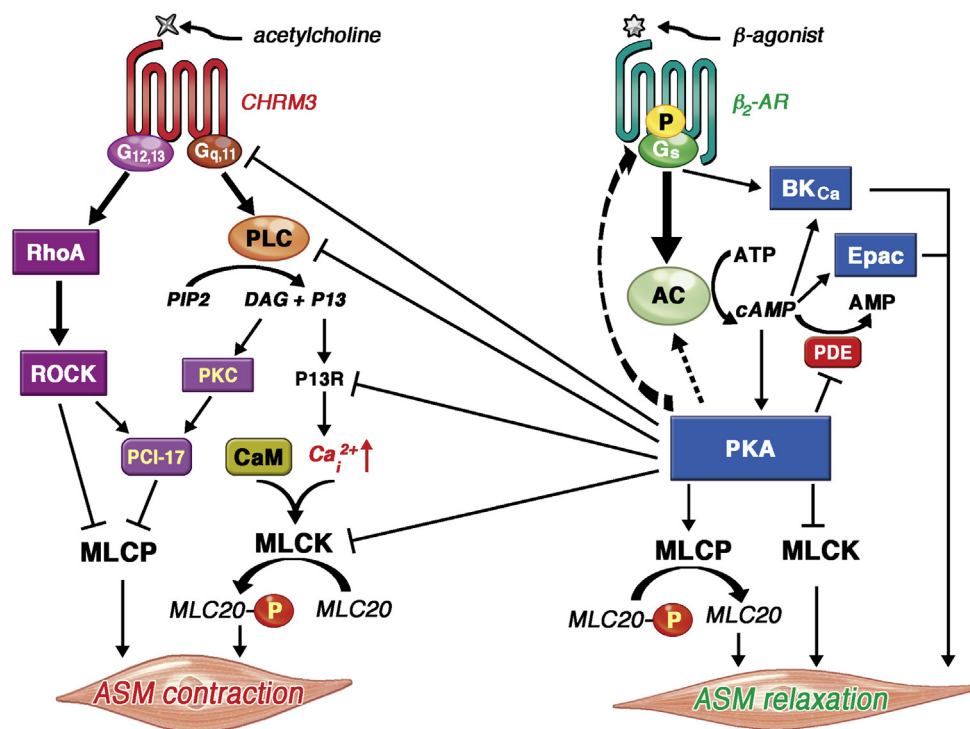


FIG 2. Contraction and relaxation signal transduction in ASM. (*Left*) Binding of acetylcholine to its receptor CHRM3 coupled to $G_{q,11}$ activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3). IP3 activates its receptor to increase cytosolic Ca^{2+} . Ca^{2+} associates with calmodulin (CaM) to activate MLCK. MLCK-mediated phosphorylation of MLC20 enables actin to activate the myosin ATPase activity required for cell contraction. DAG activates PKC, which phosphorylates CPI-17 to inhibit MLCP and favor contraction. CHRM3 also couples to $G_{12,13}$, which activates RhoA and its downstream effector ROCK. MLCP activity is suppressed by ROCK. (*Right*) β -Agonists bind to the β_2 -AR and activate G_s , which stimulates AC. AC catalyzes cAMP formation, which activates PKA and exchange protein directly activated by cAMP. PKA phosphorylates several proteins including $G_{q,11}$, PLC, and IP3 to limit Ca^{2+} mobilization, and inhibits MLCK and phosphodiesterases (PDEs), which hydrolyze cAMP to AMP. PKA stimulates MLCP to reduce phosphorylated MLC20. G_s directly and cAMP through PKA activate large conductance BK_{Ca} channels. All these events result in muscle relaxation. Negative feedback through PKA (dotted arrows) includes phosphorylation of β_2 -AR, G_s , and AC.

Contractile dysfunction in asthma

Complex mechanisms regulate the activities of MLCK and MLCP as well as the function of other kinases and phosphatases that directly or indirectly modify MLC20 phosphorylation³² and the accessory proteins caldesmon and calponin.³³ For example, stimulation of the RhoA-Rho kinase (ROCK) pathway by activation of $G_{12/13}$ protein coupled to CHRM3 (Fig 2) maintains ASM tone through stabilization of myosin filaments³⁴ and phosphorylation and consequent inhibition of MLCP.³⁵ This results in persistent phosphorylation of MLC20 independent of changes in $[Ca^{2+}]_i$ or Ca^{2+} sensitization. ROCK abundance in ASM is elevated in subjects with asthma versus subjects without asthma,³⁶ whereas the amount of p116^{Rip}, an inhibitor of RhoA-ROCK signaling, is reduced.³⁷ Human lung tissue slices exhibit augmented bronchoconstriction when they are exposed to TGF- β 1, an immunomodulator elevated in airways of patients with asthma. This effect is associated with increased MYPT1 phosphorylation mediated by the Smad3-ROCK-dependent pathway.³⁸ Therefore, enhanced ROCK activity may represent a target for asthma therapy. Today, the ROCK inhibitors, fasudil and ripasudil, are used to prevent cerebral vasospasm and to treat ocular hypertension, respectively, in Japan.

Within the described molecular design of MLC20 phosphorylation status, elevated muscle tone observed in asthma might result from alterations of the activity of MLCK and/or MLCP in response, for example, to inflammatory molecules rather than to an intrinsic alteration of myosin itself. The ASM cell shortening velocity in ragweed pollen-sensitized dogs is enhanced, a phenomenon that correlates with augmented phosphorylated MLC20 and increased activity of MLCK.³⁹ Similar results were observed using ASM from human endobronchial biopsies of subjects with asthma versus normal subjects.²⁹ Moreover, some studies indicate that neither the intrinsic contractility⁴⁰ nor the Ca^{2+} homeostasis⁴¹ seem to be different in human ASM cells from subjects with asthma compared with normal subjects. This suggests that in asthma, a normal ASM might operate within an otherwise abnormal environment⁴² of inflammation and/or mechanical distortion and/or a modified relationship of the airway wall to the surrounding parenchyma.

Ca^{2+} sensitivity, defined as a change in tone at a constant $[Ca^{2+}]_i$, also affects ASM cell contraction. Contractile agonists increase sensitivity of ASM to Ca^{2+} via RhoA-ROCK activation (Fig 2).⁴³ Conversely, formoterol, a β_2 -adrenergic receptor (AR) agonist, relaxes ASM by decreasing both Ca^{2+} sensitivity and by inhibiting Ca^{2+} oscillations.^{43,44}

The main dysfunction in asthma is the exaggerated airway narrowing, that is, airway hyperresponsiveness (AHR) in response to inhaled agents that elicit ASM contraction and the characteristic symptoms of asthma. These include wheeze, cough, chest tightness, and breathlessness. As mentioned above, another functional characteristic of asthma is the inability of DI to induce bronchorelaxation. The precise mechanism of AHR and impaired DI in asthma is not fully understood, but *ex vivo* studies with human bronchial segments suggest that an overabundance of ASM mass in asthma contributes to AHR, which in turn is independent of reduced bronchodilatory response to DI.⁴⁵ Moreover, emerging evidence indicates that inflammation and allergen sensitivity are not intimately involved in the disease in a large subgroup of patients. For example, 47% of patients with mild to moderate asthma lack airway eosinophilia,⁴⁶ whereas subjects with familial eosinophilia, compared with unaffected relatives, exhibit a decreased prevalence of asthma.⁴⁷

ASM dysfunction in asthma beyond contraction

Regardless of whether the ASM in asthma exhibits an intrinsic contractile defect or not, exaggerated persistent bronchoconstriction can contribute to the phenotypic changes present in asthmatic airways, that is, ASM cell proliferation and ASM contraction.⁴⁸ Cultured human epithelial cells mechanically compressed to simulate bronchoconstriction secrete ASM cell mitogens. These include IL-6, IL-8, monocyte chemoattractant protein-1, and matrix metalloproteinase-9.⁴⁹ The deformed epithelium augments both basal (tonic effect) and histamine-induced contraction of ASM cells through the release of endothelin-1.⁵⁰ These findings suggest that independent of inflammation, a putative positive loop of muscle-induced bronchoconstriction and an altered epithelium could promote asthma progression (Fig 1).

The effect of ASM contraction was tested in a clinical trial of 48 subjects with asthma who underwent 3 MCh or control saline challenges at 2-day intervals. Significant airway remodeling was observed in the biopsies obtained 4 days after the last challenge of the MCh but not in the control group.⁵¹ This unexpected observation is at odds with a retrospective analysis of several studies of lung function baselines of patients with asthma who received at least 2 ($n = 111$), 5 ($n = 54$), or 10 ($n = 21$) provocations with MCh or allergen. The analysis demonstrated no evidence of decreased FEV₁ with repeated MCh challenges.⁵² The discrepancy in both studies might be explained by a transient rather than a persistent decline in lung function or increase in AHR.

Taken together, the information above suggests a mechanism for attenuated DI effectiveness by which activated ASM leads to less stretch and compromised relaxation for any tidal volume during asthmatic exacerbations. This could result in impaired bronchodilation and persistent compressed epithelium that, in turn, promotes further remodeling. Although there is not yet direct *in vivo* evidence, this concept adds to the need for better management of bronchoconstriction theoretically to achieve better asthma control and minimize airway remodeling.

ASM relaxation in asthma

Although bronchoconstriction itself could contribute to asthma severity and progression rather than just be a consequence of asthma, dysregulation of ASM relaxation is thought too to be

associated with AHR. In addition to activation of MLCP, other mechanisms participate in relaxing the ASM. Stimulation of the β_2 -AR, through the synthesis of cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA), is the primary pathway to induce ASM relaxation⁵³ (Fig 2). PKA phosphorylates MYPT1 and reduces ROCK activity (Fig 2).⁵⁴ Although the classic understanding of the mechanism of β_2 -AR relaxation is through cAMP-induced PKA activity,⁵⁵ relaxation of precontracted ASM strips does not correlate with cAMP levels. This is important when considering the effectiveness of therapeutics aimed at maintaining high cAMP levels (ie, phosphodiesterase inhibitors), and it suggests the presence of mechanisms of relaxation by β -adrenergic stimulation independent of cAMP formation.⁵⁶ Opening of large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels results in cell hyperpolarization and muscle relaxation (Fig 2). Electrophysiology studies show that GTP-activated $\text{G}_{\alpha\text{s}}$ stimulates BK_{Ca} channels (Fig 2) independent of and with different kinetics from that of PKA.⁵⁶

cAMP activates other effector proteins including exchange protein directly activated by cAMP and cyclic nucleotide-gated channels (CNGs). Activation of exchange protein directly activated by cAMP induces relaxation of MCh-contracted ASM independent of PKA through inhibition of RhoA and activation of Rac1, which in turn results in reduced MLC20 phosphorylation (Fig 2).⁵⁷ Moreover, the antiproliferative action of β_2 -agonists and cAMP-elevating agents such as prostaglandin (PG)E₂ is mediated by exchange protein directly activated by cAMP and independent of PKA.⁵⁸ Cyclic nucleotides (cAMP and cGMP) directly activate CNGs. These cell membrane ion channels function mainly on the olfaction and vision pathways but have been described in many cell types. CNGs allow influx of extracellular Na^+ , K^+ , and Ca^{2+} , causing cell depolarization and potential signaling processes.

Spontaneous Ca^{2+} oscillations are present in ASM and correlate with asthma severity.²⁵ Formoterol, a long-acting β_2 -agonist, induces relaxation of histamine-contracted airways in human lung slices by reducing both Ca^{2+} sensitivity (at low doses) and the frequency of Ca^{2+} oscillations (at higher doses).⁴³ TGF- β_1 attenuates cAMP levels⁵⁹ and decreases the relaxing response of ASM to β_2 -agonist. This suggests a potential mechanism of β_2 -agonist hyporesponsiveness in asthma. Furthermore, the amount and activity of phosphodiesterase 4D, which catalyzes the degradation of cAMP (Fig 2), is about 2-fold greater in asthmatic versus nonasthmatic ASM cells,⁶⁰ impairing the action of β_2 bronchodilators.

Rhinovirus infection induces asthma exacerbations and can result in desensitization to β_2 -agonist therapy, allegedly due to internalization of the β_2 -AR on ASM cells.⁶¹ This effect is mediated by a glucocorticosteroid-sensitive increase in viral RNA in infected epithelial cells, which activates Toll-like receptors (TLRs) on the ASM cells to promote autocrine production of cyclooxygenase (COX)-2-induced PGE₂, PGI₂, and PGF₂ α by ASM cells.⁶² PGE₂⁶³ as well as PGI₂ and PGF₂ α ⁶² desensitize the β_2 -AR. These findings are consistent with the greater elevation of COX-2 mRNA and protein and of PGE₂ secretion observed in ASM cells derived from subjects with asthma compared with subjects without asthma when they are stimulated with IL-1 β , TNF- α , and IFN- γ .⁶⁴ It also indicates a potential use of COX-2 inhibitors to restore β_2 -agonist efficacy on the ASM cells of subjects with asthma during a viral infection.

Role of the ASM in airway remodeling

Airway remodeling refers to the pathologic structural alterations of airway wall thickening and mucus plugging originally described in severe asthma almost a century ago.⁶⁵ Through improved characterization and quantification, the range of structural changes encompasses increased ASM mass (Fig 1) due to cell hyperplasia and hypertrophy,⁶⁶ thickening of the basement and subepithelial membranes and the epithelium, mucus gland hypertrophy, fibrosis with altered extracellular matrix (ECM) composition and deposition, and vascular and lymphatic alterations.^{67,68} Advances in understanding the underlying complex mechanism(s) of airway remodeling are limited by the progression and longitudinal aspects of the remodeling process.⁶⁹ Remodeling is an early event in the natural history of asthma, because an overabundance of ASM is present in biopsies of children with asthma (occupying 27% of subepithelial tissue),⁷⁰ similar to the magnitude observed in adults with asthma (18%-40%).^{71,72}

ASM cells exhibit both a proliferative/secretory and a contractile phenotype *in vitro*⁷³ (Fig 1). However, a phenotypic shift *in vivo* from an ASM contractile to a proliferative/secretory phenotype is not yet demonstrated. *In vitro*, ASM cell proliferation is stimulated when cells are cocultured with epithelial cells or by exposure to epithelial-derived conditioned media.⁷⁴ Under similar conditions, the contractile phenotype is reduced, as assessed by a decrease in histamine-induced Ca^{2+} mobilization and force production. These changes are associated with decreased gene expression of smooth muscle α -actin and calponin and in myocardin, the master regulator of contractile apparatus proteins. The decreased $[\text{Ca}^{2+}]_i$ is accompanied by an increase in cAMP within ASM cells mediated by COX-1 and PGE2.⁷⁴ This effect is consistent with the muscle-relaxing action of β_2 -agonists through increased intracellular cAMP and activation of its downstream effectors.⁵³

Ex vivo, ASM cells from subjects with asthma proliferate more rapidly than those from subjects without asthma.⁷⁵ A plethora of studies show that various inflammatory mediators that are elevated in asthma stimulate ASM cells to proliferate in culture.⁷⁶ Moreover, in cocultures, house dust mite–exposed epithelial cells increase the proliferation of asthmatic but not of nonasthmatic ASM cells through an epithelial protease-activated receptor-2–dependent production of leukotrienes C4 and augmented expression of the leukotriene receptor CysLTR1.⁷⁷ However, several studies of ASM proliferation using bronchial biopsies of the central airways give conflicting results in asthmatic compared with nonasthmatic controls. This might be due to different technicalities and procedures, introduced artifacts, or unknown use of medications. Moreover, studies performed with intact ASM tissue demonstrate that the proportion of proliferating ASM cells is similar in asthmatic and control subjects, and it is not related to the ASM layer thickness or the size and number of ASM cells in fatal asthma, which exhibits marked eosinophilic airway inflammation.⁷⁸ This suggests that the increased smooth muscle in asthma over time is stable and independent of inflammation. Further studies are necessary to address whether ASM remodeling is stable and/or dynamic by determining the variation in proliferation of ASM cells over time and understanding the apparent stability/slow turnover of ASM cell populations. Also, the relative lack of impact of the duration of asthma on ASM thickening needs to be explored.

It is believed that airway remodeling leads to altered airway responsiveness and airflow obstruction. As discussed above, bronchoconstriction compresses the epithelium and results in more ASM mass and increased contraction, which, in turn, can further increase the degree of constriction. This suggests the presence of a potential positive feedback loop of bronchoconstriction in asthma that involves remodeling of the ASM. Analysis of 42 ASM-specific proteins present in the proteome of whole human intrapulmonary airways (third to fifth branching generation) shows distinct clustering of asthmatic ($n = 12$) and nonasthmatic ($n = 18$) subjects and an enrichment of components of the actin cytoskeleton and focal adhesions in the former.⁷⁹ Among them is zyxin, an actin regulator that stabilizes the ASM contractile apparatus and is increased in the ASM of subjects with fatal asthma.⁸⁰ Analysis of the total 2272 proteins detected in the airway proteome does not reveal significant separation of asthmatic from control subjects, indicating that specific changes occur within the ASM cell compartment.

The deposition and general profile of ECM proteins are altered in asthma and contribute to airway remodeling (Fig 1). Collagen I, III, and V, fibronectin, hyaluronan, laminin $\alpha 2/\beta 2$, tenascin, and versican are increased, whereas collagen IV and elastin are decreased in asthmatic versus nonasthmatic subjects.^{81,82} ASM cells from asthmatic subjects produce more collagen I and perlecan in culture than do cells from healthy subjects. These matrix proteins lead, in turn, to a more proliferative ASM cell phenotype.⁸³ Moreover, rhinovirus infection of ASM cells stimulates differential deposition of ECM components from nonasthmatic (fibronectin and perlecan) versus asthmatic (fibronectin and collagen) donors through activation of TLR-3 and TLR-7/8.⁸⁴ Cell migration of virus-naïve nonasthmatic ASM cells seeded onto asthmatic-produced ECM is greater than onto nonasthmatic-produced ECM, and migration is further enhanced onto rhinovirus-modulated ECMs. This suggests that differential ECM composition and migration of ASM cells in asthma may play a role in the remodeling of the airway.

In addition, the ECM serves as a reservoir for secreted factors and mitogens, which can be released on degradation/reconstruction of the ECM within the tissue. Fibulin-1C is an ECM glycoprotein that interacts with fibronectin and is increased in the serum and airways of asthmatic versus nonasthmatics subjects.⁸⁵ TGF- β stimulation of asthmatic but not of nonasthmatic ASM cells induces expression and deposition of fibulin-1C onto the ECM. Cell proliferation and migration also are augmented by TGF- β , but only proliferation is dependent on fibulin-1C.⁸⁵

Effect of genetic and epigenetic modifications on ASM

Asthma has a strong genetic component. Efforts to discover asthma genetic risk factors using linkage analysis or candidate-gene association studies were mostly disappointing because very large genetic effects do not exist for asthma, sample sizes were small, or few genetic markers were tested. However, genome-wide association studies (GWASs) successfully genotype thousands of individuals for hundreds of thousands of genetic variants. Analysis of 25 GWASs of asthma published between 2007 and 2016 show that 28 (of 31) variants of European ancestry were reproducible among approximately 500,000 individuals from the UK Biobank, indicating that the variants are true positives.⁸⁶ The

UK data set contains more than 44,000 individuals with self-reported doctor-diagnosed asthma. Examples of some target genes of asthma risk variants found in this and other studies include HLA-DQA1 (MHC, Class II, DQ Alpha 1), HLA-DQB1, IL-1 receptor like 1, IL-6 receptor, TLR1, IL-13, SMAD3, IL-33, and orosomucoid-like 3/GSDMB.⁸⁶⁻⁸⁹

A study of a 4-generation Indian family with asthma identifies a missense variant in the olfactory receptor *OR2AG2* gene that segregates with disease risk in all affected individuals, is absent in unaffected subjects, and was validated in an asthma pediatric cohort of different ethnicity.⁹⁰ About 80% of the patients with asthma carry a copy of the disease allele, and they show hyposmia compared with controls. This is consistent with a predicted loss of function of the *OR2AG2* gene in carriers of the variant and the observed decrease in *OR2AG2* mRNA levels in asthmatic versus control lungs. Several olfactory receptors and their downstream effectors are present outside the nose (see below). *OR2W3*, *OR1D2*, and *OR2AG1*, a paralog of *OR2AG2*, are expressed in ASM. Activation of *OR2AG1* inhibits histamine-induced contraction of ASM cells through an adenylyl cyclase (AC)/cAMP-dependent pathway of extracellular Ca^{2+} influx and opening of CNG channels, a new molecular chemosensory mechanism in these cells.⁹¹ Perfumes can exacerbate asthma in some patients. Stimulation of *OR1D2* with bourgeonal, a synthetic odor used in perfumes, leads to ASM cell contraction and release of IL-8 and GM-CSF,⁹¹ 2 cytokines involved in airway inflammation in asthma. The effects of *OR2AG1* and *OR1D2* occur without affecting ASM-cell proliferation or death.⁹¹

Most variants are within noncoding regions and have unknown function. Understanding the role of the variants and the cells/tissues in which they operate is essential for the functional interpretation of GWAS findings and disease etiology. For example, a meta-analysis reveals an enrichment of asthma risk variants for enhancers but not for promoter regions in immune cells, suggesting involvement in gene regulation.⁹² Two genetic studies of asthma and allergic diseases that also use data of tissue-specific heritability show shared loci enriched in skin and lung⁹³ as well as in lymphocytes.⁹⁴

Regardless of the central role of the ASM in asthma, “omics” data from ASM are mostly absent in available resources and thus, are missing for use in prediction algorithms. The first comprehensive study that analyzes differences in genome-wide gene expression, methylation, and contractile response between asthmatic and nonasthmatic ASM cells exposed to IL-13 and/or IL-17A shows enrichments for asthma-associated genes among IL-13- and/or IL-17A-responsive genes and for GWAS variants associated with childhood-onset asthma.⁹⁵ Interestingly, many of the asthma variants associated with AHR have *P* values that precluded genome-wide significance but may reflect the overlooked ASM component of asthma.

Despite the strong genetic predisposition, asthma genetics can “explain” a fraction of the total phenotypic variance of disease. Asthma has also a strong environmental component, which is demonstrated by the role of early-life exposures (including in uterus) and the differences in asthma prevalence among countries with different Westernized lifestyles. Cell adaptation to environmental signals (ie, Gene × Environment interactions) is mediated by epigenetics mechanisms, which are heritable changes in gene activity independent of alterations in DNA sequence. These mechanisms include histone modifications (acetylation, methylation, phosphorylation), DNA methylation (of cytosine at CpG

islands), and noncoding RNAs (such as microRNA [miR] and short interfering RNA).⁹⁶ Epigenetic alterations are dynamic, regulated, and cell-type specific.

Acetylation of histones by histone acetyltransferases is associated with activation of gene transcription, whereas deacetylation mediated by histone deacetylases associates with suppressed gene expression. Asthmatic airways versus controls have increased histone acetyltransferase activity and reduced histone deacetylase activity.⁹⁷ Moreover, the increased expression in ASM of the chemoattractant for eosinophils, eotaxin,⁹⁸ neutrophils, CXCL8,⁹⁹ and mast cells and T lymphocytes, CXCL10,¹⁰⁰ is mediated by recruitment of histone acetyltransferases to their respective gene promoters. Trichostatin A, a histone deacetylase inhibitor, prevents airway constriction of lung slices by reducing Ca^{2+} mobilization in ASM.¹⁰¹ Some asthma drugs target epigenetic changes through effects on histone-modifying enzymes. For example, salmeterol, salbutamol, fluticasone, and dexamethasone each repress TNF- α -induced eotaxin transcription in ASM by inhibition of histone acetylation.¹⁰²

There are few large-scale epigenome-wide analyses of ASM partially because of the limited availability and accessibility of ASM as a source of DNA and RNA. A study using ASM cells from healthy nonasthmatic, nonsevere, and severe asthmatic (*n* = 5 each) subjects identified more than 12,000 differentially DNA methylated positions that cluster in distinct patterns based on asthma diathesis and disease severity.¹⁰³ Differential methylation correlates with changes in expression of mRNA associated with proliferation, endocytosis, contraction, and proinflammatory pathways in ASM from severe asthmatic versus healthy subjects and with proliferation and apoptosis pathways in ASM from non-severe versus healthy subjects. More than 100 differentially methylated positions involve altered expression of miRs.¹⁰³

Noncoding RNAs are not translated. The most widely studied noncoding RNAs are miRs, which are short noncoding RNAs that bind to the mRNAs of their target genes to reduce their stability and/or inhibit translation from those mRNAs. miRs regulate many cell functions including proliferation, differentiation, migration, and apoptosis. The abundance of miR-139-5p is decreased in ASM cells of asthmatic subjects and in ASM cells of nonasthmatic individuals exposed to IL-1 β , TNF- α , and IFN- γ ,¹⁰⁴ cytokines that are elevated in severe asthma. miR-139-5p inhibits ASM proliferation and promotes apoptosis through downregulation of Brg1, a chromatin remodeling factor, and inhibition of the Akt/mammalian/mechanistic target of rapamycin pathway. The let-7 miR family is one of the most abundant in the lungs.¹⁰⁵ Expression of let-7a miR is altered in asthma, allergic rhinitis, and atopic dermatitis¹⁰⁶ as well as in malignancies and Alzheimer disease. For example, let-7a is downregulated in transbronchial lung biopsies from severe but not mild asthmatic or nonasthmatic subjects,¹⁰⁷ in bronchoalveolar lavage fluid exosomes of those with mild asthma,¹⁰⁸ and in ASM of asthmatic subjects.¹⁰⁹ Moreover, upregulation of let-7a (via transfection with let-7a mimics) enhances apoptosis and suppresses proliferation of asthmatic ASM cells,¹⁰⁹ the latter mediated through inhibition of STAT3 mRNA and protein expression. Exhaled breath condensates of subjects with asthma contain extracellular vesicles loaded with miRs, the signature of which differ from that from subjects with no asthma.¹¹⁰ The contribution of the ASM as a source of exosomes in the condensates remains to be established.

DNA methylation and miRs are promising in biomarker discovery and in clinical applications for diagnosis and/or

response to treatment as in the case of other complex diseases such as cancer.¹¹¹

Inflammatory and immunomodulatory functions of the ASM cell

A traditional view of the ASM is that it is shielded by the intact epithelial cell layer from direct interaction with inhaled particles/allergens and pathogens. However, the ASM does respond to agents that penetrate the epithelial layer (eg, inhaled particulate matter enters the blood stream) or infection of the submucosal layer, which triggers an inflammatory reaction that causes changes in the ASM cell contractile behavior, gene expression, cell growth, and capacity to synthesize and secrete active mediators and specific components of the ECM (Fig 1).

ASM cells in culture produce and release chemokines, cytokines, and growth factors in response to IL-1 β , IL-13, or TNF- α stimulation. Moreover, ASM cells exhibit proinflammatory and immunomodulatory functions³ by secreting IL-13, IL-5, IL-8, and other compounds that favor cell proliferation and recruitment of inflammatory cells such as mast cells and T lymphocytes.¹¹² Isolated ASM cells from asthmatic subjects have increased capacity to release proinflammatory chemokines and matrix proteins, which could contribute to enhanced cell proliferation⁸³ and bronchial inflammation.¹¹²

The relaxing function of ASM can be perturbed also by inflammatory mediators. For example, IL-1 β decreases the response of cultured ASM cells to isoproterenol, a β_2 -agonist, without affecting β_2 -AR numbers or the activities of AC or PKA.¹¹³ IL-1 β also attenuates the ability of PGE2 to decrease cell stiffness.¹¹³

THERAPEUTIC TARGETING OF ASM IN ASTHMA

β_2 -AR agonists and glucocorticoids are central to appropriately treat asthma. However, the β_2 -AR may be downregulated when chronically stimulated with β_2 -agonists, causing a loss of therapeutic benefit. Moreover, exposure to the inflammatory cytokines IL-1 β and IL-13 reduces β -adrenergic responsiveness of human ASM cells *in vitro*.^{114,115}

The pathways that couple G_q (to increase IP3 and elevate Ca^{2+}) and G_s (to increase cAMP to drive relaxation) are well studied. Emerging receptor biology and pharmacology of uncharacteristic G protein-coupled receptors (GPCRs) as well as of non-GPCR-dependent cascades in ASM have potential as new target for bronchodilation.¹¹⁶⁻¹¹⁸ Sensory GPCRs, including bitter taste (T2R), olfactory, and photo receptors, are widely expressed throughout the body, including the ASM.¹¹⁹ Activation of these receptors in ASM induces mostly bronchodilatation through mechanisms divergent from those used for tasting in the tongue, smelling in the nasal epithelium, or vision in the retina.

The human genome encodes 25 different T2Rs, of which 6 subtypes are functionally expressed in the ASM, wherein their activation induces muscle relaxation even in the presence of β_2 -AR tachyphylaxis. Stimulation of T2Rs evokes Ca^{2+} mobilization, which activates BK_{Ca} channels, leading to cell membrane hyperpolarization. However, other mechanisms of reduced contractility are proposed.⁴ T2Rs also inhibit ASM-cell proliferation,¹²⁰ undergo limited homologous desensitization, and their function is maintained in subjects with asthma.¹²¹ The effect of T2Rs in preventing ASM contraction is selective because a

particular T2R subtype opposes contraction induced by stimulation of certain procontractile GPCRs.¹²² Because many T2Rs are broadly tuned (a given subtype detects various bitter compounds) and they are widespread in the body including the gastrointestinal tract,¹²³ nose,¹²⁴ and immune cells,¹²⁵ off-target effects on T2Rs may play a role in the side effects of drugs in clinical use that taste bitter. This opens possibilities on repurposing some medicines to treat asthma. Identification of endogenous ligands of T2Rs and development of receptor agonists with high specificity, sensitivity, and selectivity for a specific tissue/cell remain challenging tasks.

With more than 370 genes or pseudogenes, the olfactory receptors is the largest family in the human genome. Many olfactory receptors are orphan, with no known ligand. Various odorants increase Ca^{2+} in ASM cells, leading to cell contraction (see above) or relaxation. Stimulation of the OR2W3 receptor with nerol, for example, causes bronchodilation through a complex mechanism that is independent of AC/CNG channels, PKA, or opening of BK_{Ca} channels but involves the store-operated calcium entry pathway and cell membrane depolarization, Ca^{2+} -activated chloride channel TEM16A, and the cystic fibrosis transmembrane conductance regulator.¹²⁶ This signaling cascade is different from those of the β_2 -AR and T2Rs. Stimulation of OR51E2 with acetate or propionate decreases cytoskeletal remodeling of ASM and inhibits cell proliferation of ASM cells from both nonasthmatic and asthmatic donors,¹²⁷ effects that alleviate airway obstruction. Ligands of OR51E2 are produced by the gut (and possibly by the lung microbiota), reinforcing possible benefits of dietary fibers. Further studies on the effects of agonists of OR51E2 and OR2AG1 or OR1D2 antagonists in models of asthma and allergy are warranted.

Photo-relaxation of ASM is stimulated by blue light, mediated by opsin 3 receptor, and uses the canonical G_s -AC-cAMP-PKA pathway to activate BK_{Ca} channels.¹²⁸ Costimulation of opsin 3 and β_2 -AR leads to an additive relaxing effect. However, there is functional heterodesensitization of opsin 3 with β -agonists, and the identity of endogenous chromophores and ligands remains unknown.

GABA (γ -aminobutyric acid), the major inhibitory neurotransmitter in the central nervous system, stimulates GABA_A (ligand-gated ionotropic) and GABA_B (metabotropic GPCR) pentameric receptors present in ASM. Activation of GABA_A receptors (GABA_ARs) alters membrane potential, generates chloride currents, relaxes precontracted ASM, and potentiates the action of isoproterenol.¹²⁹ This effect is mediated by the α_4 and α_5 subunits of GABA_AR, which minimizes potential side effects (sedation, hypnosis) driven by distinct GABA_AR subunits expressed in the nervous system. Using mouse lung slices, activation of GABA_AR opposes airway narrowing by attenuating Ca^{2+} elevation and Ca^{2+} oscillations induced by procontractile agonists.¹³⁰ The glycine receptor chloride channel also changes membrane potential and attenuates contraction in ASM.¹³¹ However, stimulation of GABA_B receptors on ASM, which couples to G_i , results in increased IP3 and Ca^{2+} mobilization. These effects depend on activation of phospholipase C β by the $\beta\gamma$ subunits of G_i ¹³² and may facilitate activation of G_q signaling. Consistently, in a clinical trial of subjects with asthma, baclofen, a GABA_B receptor agonist, worsens bronchial responsiveness to methacholine, a G_q -coupled GPCR agonist.¹³³

N-methyl-D-aspartate receptors (NMDARs) are gated nonspecific cation channels abundant in nerves that are activated by

TABLE I. Proposed areas of ASM research and their opportunities and difficulties

Area of research	Opportunities and difficulties
Biology of postnatal ASM in health	Define potential advantages of ASM function in normal state. Catalog smooth muscle cell heterogeneity/subtypes according to their location in the respiratory tree using single-cell RNA sequencing approaches.
Role of ASM in airway inflammation and remodeling	Establish whether ASM contributes quantitatively to asthmatic airway inflammation. Develop interventions that limit ASM-cell proliferation and/or enhance ASM-cell apoptosis. Discover pathways to restore ASM bronchodilating properties.
BT-induced ASM ablation	Identify the population most likely to benefit from BT. Determine the long-term effects of ASM loss on lung function and general homeostasis. Define predictors of BT response. Develop BT protocols optimized to target specific airways or elements of airway remodeling. Understand the molecular and cellular mechanisms of the beneficial effects of BT.
Improved preclinical models	Link biochemical/mechanical events in cells, organoids, lung-on-a-chips, tissues, and animals to clinically relevant (patho)physiology in humans.
Clinical studies	Design trials that include assessment of the ASM along with disease heterogeneity, use of medications, and genetic variability.

various agonists including glutamate. NMDAR activation mediates ASM contraction through sustained elevation of Ca^{2+} , which requires extracellular Ca^{2+} and glycine, an NMDAR coagonist.¹³⁴ NMDAR expression in cultured ASM cells is enhanced by exposure to TNF- α , associates with differential expression of NMDAR subunits, and enhances Ca^{2+} mobilization and contractility. Paradoxically, stimulated NMDAR results in bronchodilation of lung slices treated with TNF or from house dust mite-challenged mice, a model of acute lung inflammation and AHR.¹³⁵ This NMDAR-relaxing effect requires iNOS and COX-2 likely from the airway epithelium or lung endothelium. The presence and function of pulmonary glutamatergic signaling in modulating airway responsiveness through NMDAR in asthma remains unclear.

With increasing knowledge of the complex role of the ASM in the pathophysiology of asthma, promising therapies are evolving. Inhibition of RhoA-ROCK signaling may represent a new approach. Further studies to provide clinical data, investigate the functional significance of the RhoA-ROCK pathway, and determine the underlying mechanisms in the regulation of innate immune responses and host defense are essential.

It is feasible that the mechanical stretch of the ASM with the use of continuous positive airway pressure (CPAP) could achieve reduced airway reactivity. Small, short-term studies with adult asthmatic subjects show decreased bronchial hyperreactivity with CPAP treatment.¹³⁶ However, a larger ($n = 194$), 12-week randomized controlled trial reports that CPAP at less than 1 cm H_2O (sham) or 5 or 10 cm H_2O improved bronchial reactivity equally,¹³⁷ questioning the effectiveness of CPAP to treat asthma and suggesting that breathing heated humidified filtered air might be of benefit.

Bronchial thermoplasty (BT) was approved by the Food and Drug Administration in 2010 to treat severe asthma in adults with history of a Global Initiative for Asthma step 4 treatment consisting of maximal inhaled corticosteroids and long-acting β -agonists. During BT, radiofrequency energy-producing heat is delivered to the central airways via a bronchoscope that reduces ASM mass¹³⁸ (Fig 1). Although asthma morbidity frequently increases during the BT peritreatment period, observational studies using sham controls¹³⁹ confirm long-term improvements measured by the asthma control questionnaire, asthma quality

of life questionnaire, rescue medication use,¹⁴⁰ decrease in asthma exacerbation by 40% and hospitalization by 45%, and absence of clinical complications.¹⁴¹ The exact mechanism of action of BT is not yet established, but it is speculated that, in addition to ASM mass loss, alterations of mucus glands,¹⁴² airway epithelium,^{143,144} neuroendocrine cells,¹⁴⁵ nerves,¹⁴⁶ collagen deposition beneath the basal membrane,¹⁴⁷ number of myofibroblasts in the lamina propria,¹⁴⁸ and inflammation^{142,144,149} also play a role. However, the only randomized controlled clinical trial (NCT0222539) with 40 severe asthmatic subjects that compares immediate BT versus 6-month delay BT¹⁵⁰ shows that clinical improvement in asthma control questionnaire and asthma quality of life questionnaire scores is not associated with the more than 50% reduction in ASM abundance. Rather, the benefit is associated with the baseline serum IgE levels and eosinophil counts. Moreover, lung function, assessed by FEV₁ and bronchial hyperresponsiveness during MCh challenge, does not significantly change following BT. These results suggest that patients with severe asthma with high serum eosinophils and/or IgE levels may be more responsive to BT treatment. Studies with larger cohorts are needed to confirm this hypothesis.

A pilot study of a single session of ¹²⁹Xe MRI-guided BT shows similar short-term improvement in quality of life when compared with the standard 3-treatment-session of BT. Improvement is also associated with diminished number of adverse events.¹⁵¹ Of note, candidates for BT belong to the same group eligible for treatment with biologics. These subjects possibly could benefit clinically with BT while preventing the necessity for lifelong and expensive biological therapy. A single-center, observational study in Australia comparing BT ($n = 44$) with treatment with mepolizumab ($n = 47$), an mAb that inhibits IL-5, shows no statistically significant difference between the 2 cohorts at 12 months relative to their respective baselines in the improvement in asthma control questionnaire score and lung function, exacerbation frequency, improved short-acting β -agonist usage, and maintenance of oral glucocorticosteroids. Moreover, the benefit in subjects with asthma with greater than or equal to 300 eosinophils/ μL after BT was undistinguishable from that in subjects treated with mepolizumab.¹⁵²

Increasing number of biomarkers are part of the diagnostic tools used in clinical practice. Delivering individualized therapy

is a new benchmark in asthma treatment. However, the use of biologics, BT, and other interventional procedures requires an accurate diagnosis and precise phenotyping (and endotyping). Unfortunately, current treatments do not specifically target airway remodeling.⁶⁹

FUTURE ISSUES

Possible approaches aimed at preventing ASM dysfunction in asthma and/or restoring normal ASM structure and function could include recovering the antiobstructive effect of tidal breathing, stimulation of apoptosis of airway myocytes to alleviate remodeling,¹⁵³ and selective inhibition of serum response factor and myocardin activities in ASM cells to avert expression of genes encoding smooth muscle contractile apparatus proteins.¹⁵⁴ Moreover, actin polymerization and recruitment of the cytoskeletal proteins vinculin and paxillin to cell membrane focal adhesions are events important for the tension transmission between the contractile apparatus and the ECM during contraction.¹⁵⁵ Disruption of these processes could also be targeted to control ASM cell contraction.

An exciting study using tracheal smooth muscle strips demonstrates that combined interventions of salbutamol, a short-acting β -agonist, (0.1 nM), the ROCK inhibitor H1152 (0.1 nM), and force oscillations (delivering pressure lower than those induced by DI) synergistically relax precontracted ASM.¹⁵⁶ That is, although each intervention applied alone does not cause significant relaxation, the combination of all 3 reverses 50% to 60% of the shortening. Moreover, combining any 2 of them reverses approximately 30% of the shortening, a change that could translate into 65% to 80% reduction in airway resistance. Clinically, it will be interesting to test whether effective smooth muscle dilation can be achieved with a mechanopharmacological approach by combining small oscillation amplitudes with very low doses of a β -agonist plus a ROCK inhibitor, which would help reduce side effects and prevent tolerance. Other areas of further interest not covered here are described in Table I.

CONCLUSIONS

The ASM contributes to the multifactorial and heterogeneous pathology of asthma through mechanisms beyond the effects of bronchoconstriction. Short- and long-term duration of ASM contraction alters the mechanical properties of the airway wall, which contribute to remodeling and modulates inflammatory signaling cascades within the ASM and in other cells types (epithelium, fibroblasts, immune cells, and vasculature). Reductionist and integrated experimental approaches as well as mathematical/computational modeling are instrumental to fully understand the pathogenesis of this complex disease. The mechanisms that regulate ASM contractility in response to environmental stimuli, secreted factors from other airway cell types and/or infiltrating inflammatory cells, and from circulating mediators remain incompletely understood and constitute an exciting aspect of basic, translational, and clinical lung research.

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