Airway smooth muscle in the pathophysiology and treatment of asthma

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Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. J Appl Physiol 114: 834–843, 2013. First published January 10, 2013; doi:10.1152/japplphysiol.00950.2012.—Airway smooth muscle (ASM) is a chronic disease of the airways affecting over 24 million people in the United States (94).1 It is characterized by intermittent airflow obstruction and airway inflammation, producing symptoms of chest tightness, wheezing, and cough. Structural and inflammatory changes throughout the airway wall lead to bronchial thickening and edema as well as increased mucus production and bronchoconstriction, all of which contribute to the episodic airflow obstruction typically found in asthma (Fig. 1). In recent years, there has been much attention on inflammation in asthma, for example, whether the type of inflammatory cell predominately found in the airway denotes a specific phenotype in asthma or whether targeting antibodies, inflammatory cytokines, and inflammatory cells is helpful for the treatment of asthma (56, 96, 115). Although airway smooth muscle (ASM) has been implicated in constrictor hyperresponsiveness in asthma for decades, other important roles of ASM have recently been identified. Through impaired airway relaxation and mediation of structural changes and inflammatory signaling, the ASM cell plays multiple diverse roles in the pathophysiology of asthma. This review is meant to update practicing physicians with current knowledge about these less discussed direct effects of ASM on airway narrowing and indirect influences on airway remodeling and inflammation in asthma (Fig. 2).

FUNCTIONAL CHANGES IN ASTHMATIC ASM

ASM is most well known for its role in acute bronchoconstriction. Smooth muscle surrounds the airway in a circumferential pattern, reducing the airway luminal diameter as it contracts. It is this function of ASM that causes the acute airflow obstruction, shortness of breath, and wheezing most commonly associated with the clinical syndrome of asthma. In asthma, ASM is primed to contract, often excessively, in response to various stimuli, but in addition, it resists relaxation. These two phenomena are now discussed in turn.

Airway Hyperresponsiveness

The excessive contractile response of ASM in asthma results in inordinate bronchoconstriction and airflow obstruction in response to relatively little provocation; this phenomenon is denoted as airway hyperresponsiveness. A variety of chemical and physical stimuli can trigger bronchoconstriction (Table 1). Contractile agonists, like methacholine, can directly activate receptors on ASM cells that initiate myocyte contraction and consequent bronchoconstriction, and this is the basis of the methacholine challenge test sometimes used to diagnose asthma. Substantial respiratory heat and water loss, as occur during exercise in temperate or cold climates, can also provoke bronchoconstriction, likely mediated by contractile agonists released from mast cells or nerves exposed to a hyperosmolar milieu (4, 44). Why individuals with asthma are hyperresponsive compared with healthy individuals is complex. Contributing mechanisms include both an increased availability in asthmatic airways of contractile mediators such as histamine from mast cells (12) and increased ASM mass. Mathematical models initially proposed that excessive ASM generates abnormally increased force (77), but increased dynamic muscle stiffness (due to impaired breathing-induced muscle softening) may actually be the more important mechanism (102). Other explanations for airway hyperresponsiveness in asthma include increased vagal tone (13, 26), cytokine-potentiated increases in intracellular free calcium that enhance ASM cell contractility (51) and activation of the procontractile Rho kinase pathway (19, 124). Increased RhoA protein levels have also been identified in animal models of allergic asthma (20, 21, 123), and inhibition of the RhoA-Rho kinase pathway can prevent or reverse airway hyperresponsiveness (Table 2) (121, 122).

Impaired Relaxation

Aberrant shortening of ASM in asthma can also partly be explained by inadequate relaxation. There is no direct sympathetic innervation in human ASM (17). Additionally, β2-adrenergic receptors may become downregulated when bom-
barded frequently with β_{2}-agonist medications, in a fashion that depends in part on genetic polymorphisms (69). This phenomenon has been observed in a number of cell types, including ASM (50, 93), and may further potentiate the imbalance of autonomic neurotransmitter influences on the airway in asthma. In fact, patients with asthma can develop tolerance to β-agonist therapy (57). Inflammatory cytokines further potentiate this effect. Interleukin-1β (IL-1β), a cytokine produced by a variety of lung cells, reduces β-adrenergic responsiveness in cultured human ASM cells (79). Another important inflammatory cytokine, IL-13, has a similar effect on adrenergic receptors, albeit through a different mechanism (78). Prevention or reduction of β_{2}-adrenergic receptor desensitization with agents that preserve receptor expression and function, such as ascorbate (31) or alendronate (67), might conceivably be helpful in the treatment of asthma.

Breathing-Induced Reversal of Bronchoconstriction and Length Adaptation

Other properties of ASM function may also contribute to airway narrowing in asthma. It has long been observed that in normal people, deep breathing can reverse bronchoconstriction (3, 98). Within the lung, the airways are connected to the lung parenchyma, which is firmly attached to their adventitial surfaces. With each inspiration, the lung parenchyma surrounding each airway expands and pulls radially outward on the airway, stretching it partially during the breath. Isolated ASM that is forcibly lengthened while still contracting reduces its force of contraction (49), likely due to perturbation of actin-myosin interactions (41). Furthermore, fluctuations in the force applied to isolated contracting smooth muscle, simulating the tidal stretches that occur with breathing, cause it to relengthen, even when the mean force is held constant (33, 76, 80). As a result of these behaviors of ASM, stretching of bronchoconstricted airways by breathing in part reverses the lumenal narrowing that had been present (102). However, in individuals with asthma, the ability of deep breaths to reverse bronchoconstriction seems blunted; this might stem from increased muscle mass and increased muscle stiffness as noted above or might reflect other mechanisms that are not yet fully understood. Nonetheless, the role of airway distension during a deep breath seems certain, for pronounced bronchoconstrictor responses to methacholine, similar to those of patients with asthma, can be elicited in normal subjects when deep breathing is restricted (127). Recently, we and others have found pharmacological interventions that potentiate ASM relengthening in response to force fluctuations in vitro (32, 33, 75, 76). Pharmacologically potentiating the ability of deep inspirations to reverse bronchoconstriction might represent a novel therapeutic approach to relieve or prevent airflow obstruction in the future. Indeed, corticosteroids, a mainstay of asthma treatment, might exert some of their therapeutic effect through this mechanism (72).

Another inherent property of smooth muscle may in part explain why asthmatic airways are not as susceptible to deep breathing-induced stretch during an asthma attack. When a smooth muscle cell shortens, it adapts by reorganizing its contractile filaments in a way that will allow it to generate the same force at its new length (125). This can create a vicious

Fig. 1. Multiple mechanisms of airflow obstruction in asthma, including bronchoconstriction by airway muscle, obstruction of airflow by intraluminal mucus, and inflammation and remodeling of the airway wall. [Adapted with permission from the National Heart, Lung, and Blood Institute.]
cycle in asthma, because once ASM has shortened, it quickly becomes primed to shorten further (89). Now more severely shortened, the muscle may be stiffer and less influenced by the antibronchoconstrictor effects of deep breathing (102). In sheep trachealis, passive stiffness of ASM is also increased at shorter lengths (10). Indeed, chronic application of continuous positive airway pressure (CPAP; which presumably chronically holds airway muscle at greater length) reduced ASM contractility in ferrets (149), reduced airway hyperresponsiveness in rabbits with experimental asthma induced by ovalbumin sensitization and challenge (148), and most importantly reduced airway constrictor responsiveness in volunteers with stable asthma and normal spirometry (15). But if CPAP reduces airway hyperresponsiveness, why doesn’t the pulmonary hypertension that accompanies acute asthma attacks simply reverse the bronchoconstriction? We speculate that the pulmonary hypertension that attends acute asthma attacks may be less effective in reversing bronchoconstriction because the patients’ tidal volumes are likely reduced by the dynamic hyperinflation. If dynamic stretch is more important than mean stretch in determining overall airway smooth muscle shortening [as appears to be the case (40, 41)], then the adverse effect of pulmonary hypertension (reducing dynamic stretch) during acute bronchoconstriction might outweigh its potential beneficial effect (enhancing mean stretch). No studies have specifically addressed this balance in acute asthma to date, and the potential for CPAP treatment to reduce asthma symptoms or medication use is currently under study.

### Table 1. List of stimuli provoking bronchoconstriction in asthma

<table>
<thead>
<tr>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct stimuli</td>
<td></td>
</tr>
<tr>
<td>Cholinergic agonists (e.g., methacholine)</td>
<td>11</td>
</tr>
<tr>
<td>Histamine</td>
<td>12</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td></td>
</tr>
<tr>
<td>Leukotrienes</td>
<td></td>
</tr>
<tr>
<td>Indirect chemical stimuli</td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>138</td>
</tr>
<tr>
<td>NSAIDs</td>
<td></td>
</tr>
<tr>
<td>Tachykinins, bradykinin</td>
<td></td>
</tr>
<tr>
<td>Endotoxin</td>
<td></td>
</tr>
<tr>
<td>Allergens</td>
<td></td>
</tr>
<tr>
<td>Indirect physical stimuli</td>
<td></td>
</tr>
<tr>
<td>Exercise with heat and/or water loss</td>
<td>4, 44</td>
</tr>
<tr>
<td>Hypertoncity (hypertonic saline or mannitol)</td>
<td>102</td>
</tr>
<tr>
<td>Increased ASM mass</td>
<td></td>
</tr>
<tr>
<td>Increased vagal tone</td>
<td>13</td>
</tr>
</tbody>
</table>

ASM, airway smooth muscle.

### Table 2. Potential new therapeutic targets of ASM function in asthma

<table>
<thead>
<tr>
<th>ASM Function</th>
<th>Potential Therapy</th>
<th>Status of Therapeutic Development</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contractility</td>
<td>Inhibition of Rho kinase pathway</td>
<td>Animal models</td>
<td>124</td>
</tr>
<tr>
<td>β2-adrenergic receptor modulation</td>
<td>Adrenergic enhancement</td>
<td>Animal models</td>
<td>31</td>
</tr>
<tr>
<td>Relaxation</td>
<td>Potentiation of breathing-induced relengthening</td>
<td>In vitro animal studies</td>
<td>32, 33, 75, 76</td>
</tr>
<tr>
<td>Stiffness</td>
<td>CPAP</td>
<td>Reduction of AHR demonstrated in asthma Clinical trials re asthma control ongoing</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Reduce ASM mass</td>
<td>Bronchial thermoplasty FDA-approved 2010</td>
<td>140</td>
</tr>
</tbody>
</table>

AHR, airway hyperresponsiveness; CPAP, continuous positive airway pressure; FDA, Food and Drug Administration.

### Structural Changes in ASM in Asthma: Remodeling

Remodeling of the airway refers to pathologic changes such as increased ASM mass, basement membrane thickening, and mucus gland hyperplasia (1, 66) (Fig. 3). These are common features of asthma that contribute to airflow obstruction both by luminal encroachment and by enhancing constrictor hyperresponsiveness. In addition, bronchoconstriction per se appears to promote airway remodeling (46). Thus airway inflammation and bronchoconstriction might participate in a vicious cycle that maintains the structural abnormalities characteristic of asthma. Here we will focus on the pathophysiology of increased ASM mass in asthma.

At the outset, we should note that assessing ASM mass in living subjects is complicated by the nonrandom nature of endobronchial biopsies and the inability to sample the entire airway (61). Also, ASM abundance varies along the length of the airway and with age (63). To help create an internal reference when measuring ASM in bronchial biopsies, some studies have quantified ASM mass as a percentage of airway wall subepithelial tissue (112). Confocal bronchoscopy has recently been used to assess airway wall structure without the need for biopsy and holds promise for allowing assessment of ASM mass (97, 135).

**Increased ASM Mass in Asthma**

Increased ASM mass has been identified as a hallmark of asthma, and its abundance is particularly great in fatal (64) or severe (6) asthma. There is much debate, however, about the mechanism driving its excess accumulation. Both increased ASM cell size (hypertrophy) and cell number (hyperplasia) have been described (37, 65), with hypertrophy predominant in some subjects and hyperplasia characteristic of others. One study found that ASM hypertrophy was significantly increased in individuals with severe asthma (6), and studies in cell culture suggest that the PI3K-Akt-mTOR-p70S6 kinase pathway is involved (30, 53, 85). However, most research has focused on the mechanism of ASM hyperplasia (58). Stimuli that induce hyperplasia in cultured ASM cells include growth factors such as transforming growth factor (TGF-β1), epidermal growth factor, platelet-derived growth factor (24, 59, 103, 131, 147), and contractile stimuli acting through G-protein-coupled receptors, including histamine (105) and leukotriene D4 (104). TGF-β1 is a particularly important growth factor implicated in asthma. It is secreted by both infiltrating inflammatory cells and resident cells native to the airway (35) (including ASM; see below), and exposure to allergens increases TGF-β1 in the bronchoalveolar lavage fluid of individuals with asthma (111). Furthermore, cultured ASM cells
obtained from endobronchial biopsies of individuals with asthma demonstrate more rapid proliferation than do those from normal individuals (70). This might be explained by the absence of an antiproliferative transcription factor, C/EBPα (116). As C/EBPα also mediates the antiproliferative effect of corticosteroids in normal ASM, its reduction in asthmatic ASM may represent the underlying mechanism (116).

Another possible mechanism of increased ASM mass is the migration and differentiation of fibrocytes from bone marrow (120). Fibrocytes exposed in culture to TGF-β acquire characteristics of smooth muscle cells (120), and the number of circulating fibrocytes in the peripheral blood of individuals with asthma with chronic airflow obstruction correlates with their rate of decline in lung function over time (142). Therefore, interest has developed in TGF-β as a therapeutic target in asthma (Table 3). Animal models of allergen-induced asthma have shown significant reductions in ASM proliferation following TGF-β neutralization (88) or inhibition (83).

**ASM Role in Airflow Obstruction**

It remains unknown whether the mechanism driving ASM hyperaccumulation has a deleterious impact on airway contractile function directly. However, it is clear that excessive ASM mass (145) and airway wall thickening (which can be gauged by CT scan) (84, 100) are associated with airway hyperresponsiveness. Furthermore, asthma patients with fixed and severe airflow obstruction (postbronchodilator FEV1 < 50%) have significantly thicker airway walls than those with reversible airflow limitation (14). Those with fixed airflow obstruction also have longer disease duration (14), suggesting that airflow remodeling contributes to the decline in lung function seen in individuals with asthma. These studies indicate that ASM remodeling contributes to both transient and chronic airflow obstruction in asthma.

In light of its importance in airflow obstruction in asthma, ASM has been an important target for asthma therapies, including bronchodilators (5) and bronchial thermoplasty (140), a novel therapy for severe asthma. Bronchial thermoplasty is a bronchoscopic procedure in which the proximal airways are treated with radiofrequency current that heats the airway wall to 65°C with consequent reduction in ASM mass and some alleviation of bronchial constriction. In early trials with dogs, bronchial thermoplasty reduced airway hyperresponsiveness and ASM mass for up to 3 years; the reductions in airway responsiveness and in ASM mass were correlated, suggesting that the structural change in ASM mass could explain the functional change in constrictor responsiveness (29). A reduction in ASM was also seen in eight patients with lung cancer undergoing thermoplasty prior to lobectomy for their malignancies (90). The only randomized, double-blind, sham-controlled study in humans went on to show reduced exacerbations and health care visits and improved quality of life of individuals with asthma that received active treatment (18). The mechanism by which thermoplasty reduces ASM in the airways is not understood, although bovine ASM treated with thermal energy shows immediate loss of contractility and disruption of actin-myosin connections (36). Further study is needed to elucidate the mechanism driving the apparent beneficial effects of bronchial thermoplasty. It might also be possible to develop alternative less invasive means to ablate ASM in asthma (129).

**ASM AS A CONTRIBUTOR TO AIRWAY INFLAMMATION IN ASTHMA**

There is evidence that ASM plays a pivotal role in the airway inflammation characteristic of asthma as well. Not just a bystander in this process, ASM releases and expresses several molecular signals that contribute to bronchial inflam-
Table 4. Potential new therapeutic targets of inflammation in asthma

<table>
<thead>
<tr>
<th>Inflammatory target</th>
<th>Potential Therapy</th>
<th>Status of Therapeutic Development</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5</td>
<td>Anti-IL-5 monoclonal antibodies (e.g., mepolizumab)</td>
<td>Clinical trials</td>
<td>54, 99, 106</td>
</tr>
<tr>
<td>IL-13</td>
<td>Anti-IL-13 monoclonal antibodies</td>
<td>Clinical trials</td>
<td>108</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Anti-TGFβ antibody</td>
<td>Animal model</td>
<td>85</td>
</tr>
<tr>
<td>Eosinophils mast cells</td>
<td>Anti-IgE monoclonal antibody</td>
<td>Omalizumab FDA-approved 2003</td>
<td>130</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>Anti-IL-2 receptor monoclonal antibody (e.g., daclizumab)</td>
<td>Clinical trials</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Anti-CD4 monoclonal antibody (e.g., keliximab)</td>
<td>Clinical trials</td>
<td>72, 73</td>
</tr>
</tbody>
</table>

IL, interleukin; TGF, transforming growth factor; IgE, immunoglobulin E.

Cytokines

Interleukin-5. Interleukin (IL)-5 is a T-helper type 2 (Th2) cytokine. It is crucial in the survival, migration, and activation of mast cells and eosinophils, key inflammatory cells in allergic asthma (91). Although T lymphocytes are thought to be the main producers of IL-5 in the lung, human ASM cells in culture release IL-5 when exposed to serum from individuals with atopic asthma (52). Increased levels of IL-5 also appear in the bronchoalveolar fluid of individuals with atopic asthma (134), and animal studies have highlighted the role of IL-5 in allergic airway inflammation and bronchoconstriction (39, 55, 82). In addition, IL-5 deficiency inhibited airway remodeling in an ovalbumin sensitization mouse model of asthma (22). These findings led to initial interest in IL-5 modulation therapy for asthma, although the clinical effectiveness of this approach remains controversial (91) as studies have shown promise in reducing exacerbations in patients with severe eosinophilic asthma but not in consistently improving symptoms or lung function (54, 99, 106).

IL-13. Another important cytokine in asthma is IL-13. It, like IL-5, also induces eosinophilia in addition to goblet cell hyperplasia and immunoglobulin class switching to IgE (101). In mice, genetic deletion (141) or neutralization (48, 144) of IL-13 ablates allergen-induced experimental asthma. IL-13 receptors have been identified on ASM, and IL-13 induces proliferation and hypercontractility in human ASM (95, 114). Eotaxin, discussed below, is released from ASM cells in response to IL-13 (107). Various human IL-13 antibodies have been developed for the treatment of asthma; however, studies to date have variously shown little clinical benefit (108) or only mild improvement in prebronchodilator FEV₁ without reduction of asthma symptoms (25). This suggests that blocking IL-13 alone is not sufficient to prevent asthma manifestations in humans.

TGF-β. ASM cells synthesize and release TGF-β₁, whose role in airway remodeling in asthma is discussed above. Once secreted, TGF-β₁ induces ASM to produce procollagen I, a main component of fibrotic tissues (27, 87). TGF-β activation by human ASM cells may be induced by contractile agonists such as methacholine (133), and activated TGF-β is increased in individuals with asthma (118, 136). TGF-β₁ has also been implicated in the migration of mast cells to the lung and in the regulation of lung mast cell function. In a mouse model of allergic asthma, blocking TGF-β activation reduced airway responsiveness (132), and in airway biopsies from individuals with asthma, the number of mast cells infiltrating the ASM layer correlates with TGF-β₁ expression (7). TGF-β also induces differentiation of the myofibroblast, an intermediate cell phenotype between fibroblasts and ASM cells thought responsible for fibrosis in the lung (117). Blocking TGF-β can reduce airway remodeling, including ASM proliferation, in mice (88). However, clinical trials of TGF-β blockade in asthma have not yet been reported.

Other Immunomodulatory Factors

Eotaxins and eosinophils. ASM can produce chemokines, such as eotaxin, that recruit eosinophils from the systemic circulation to the lungs (43). Eotaxin is upregulated in asthma, and eosinophils collected from individuals with allergies and asthma have an enhanced response to eotaxin compared with those from normal subjects (119). Various inflammatory cytokines such as IL-1β and tumor necrosis factor (TNF-α) induce eotaxin production and release by cultured human ASM (23, 92). ASM expresses cell adhesion molecule receptors that bind eosinophils and other inflammatory cells (62). A sizable number of individuals with asthma with an eosinophilic phenotype may especially respond to specific treatments such as anti-IgE,
anti-IL-5, and anti-IL-13 therapies (126, 146). In our opinion, the interactions between eosinophils and ASM merit further investigation.

Mast cells. Mast cells are also important in the pathophysiology of asthma. Activated mast cells release histamine and other inflammatory mediators that cause bronchoconstriction and the recruitment of immune cells to the airway (109). Mast cells accumulate in the ASM layer of the airways in individuals with asthma (2, 8), including those with both eosinophilic and noneosinophilic bronchial inflammation (9). In culture, human ASM cells attract mast cells by producing cytokines such as TGF-β1 (7). An adhesion molecule expressed by the mast cells then allows them to adhere to ASM cells directly (150). This adhesion interaction is further enhanced in ASM cells from individuals with asthma (45). Airway myocyte cells can promote survival of mast cells, thereby augmenting the opportunity for persistent airway mast cell activation (60).

T lymphocytes. CD4 + T lymphocytes have also been identified within ASM bundles along asthmatic airways. In fact, T lymphocytes burden within the ASM layer of individuals with asthma was correlated with severity of disease (110). Parallel to its interactions with other inflammatory cell types described above, ASM secretes CCL5 or RANTES, a chemokine that signals T lymphocyte migration to the lung (68). Likewise, adhesion between these two cell types has also been described, although this has not yet been confirmed in asthma specifically (81). And although ASM is not usually thought of as an antigen-presenting cell, there is evidence that ASM cells not only express major histocompatibility complex class II molecules but also are capable of presenting antigens. For example, ASM can present superantigens from staphylococcus to T helper lymphocytes, resulting in their nonspecific activation (139). Whether this is a significant mechanism of antigen presentation in vivo or in individuals with asthma, however, has yet to be determined.

GENETIC CONTRIBUTORS TO ASM CHANGES: GENE POLYMORPHISMS

Although many genes can affect airway hyperresponsiveness and ASM function, variations in only a few genes are thought to influence asthma through their effects on ASM (Table 5). For example, polymorphisms in the β2-adrenergic receptor gene have been identified (113, 128, 143), and patients with asthma with the Arg16/Arg16 genotype exhibit reduced receptor gene expression (139). Whether this is a significant mechanism of antigen presentation in vivo or in individuals with asthma, however, has yet to be determined.

CONCLUSION

ASM is a crucial player in the pathogenesis of asthma. Its plays a hyperresponsive contractile role in the acute airflow limitation that is characteristic of asthma and exhibits impaired relaxation. It also modulates the chronic airway remodeling and inflammation that are essential features of the asthma syndrome. For these reasons, ASM should be considered as an important therapeutic target. Questions ripe for study include: How can the persistent shortening of ASM in asthma be prevented? How can ASM mass be controlled or reduced beyond bronchial thermoplasty? How important quantitatively is the ASM contribution to asthmatic airway inflammation, and can this contribution be targeted specifically by reducing ASM mass or by novel pharmacological interventions?

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: D.C.D. and J.S. prepared figures; D.C.D. drafted manuscript; D.C.D. and J.S. edited and revised manuscript; D.C.D. and J.S. approved final version of manuscript.

REFERENCES


