

Point:Counterpoint: Alterations in airway smooth muscle phenotype do/do not cause airway hyperresponsiveness in asthma

POINT: ALTERATIONS IN AIRWAY SMOOTH MUSCLE PHENOTYPE DO CAUSE AIRWAY HYPERRESPONSIVENESS IN ASTHMA

Summary. Airway hyperresponsiveness and obstruction are cardinal features of asthma. Compelling evidence now suggests that the key features of asthma in part are due to intrinsic differences in airway smooth muscle (ASM) function. Inflammatory mediators alter ASM phenotype, which directly modulates ASM hyperresponsiveness and promotes airway remodeling that further contributes to amplified airway obstruction. For decades, the most effective and common therapies in the management of asthma have focused on bronchodilation and reversal of ASM shortening. Accordingly, alterations in the phenotype of ASM, the pivotal cell regulating bronchomotor tone, fundamentally contribute to airway hyperresponsiveness in asthma.

Asthma, a disease characterized by airway inflammation, hyperresponsiveness, and reversible airflow obstruction, remains a highly prevalent respiratory disease accounting for profound morbidity. Airway hyperresponsiveness and reversible airflow obstruction are primarily due to shortening of ASM, the pivotal cell responsible for regulating bronchomotor tone. Over the past 20 years, evidence has accumulated that airway hyperresponsiveness results from intrinsic abnormalities in the ASM of people with asthma that modulate excitation-contraction coupling mechanisms, enhance ASM cell proliferation, alter the production of extracellular matrix proteins, and enhance the production of inflammatory chemokines and cytokines. Collectively these phenotypic alterations in the ASM cell may be responsible for many of the pathologic properties of the asthmatic airway and are likely to be the primary mechanism for airway hyperresponsiveness.

A substantial body of evidence indicates that asthmatic ASM manifests enhanced shortening and contractility *in vitro* compared with ASM from normal subjects. Several studies have directly compared the *in vitro* contractility of ASM cells and tissues isolated from asthmatic and normal subjects, and found that the shortening or contraction of ASM isolated from asthmatic subjects is greater than that from normal subjects. Bronchi prepared from surgical specimens from asthmatic lungs have also been found to exhibit increased sensitivity to adenosine compared with those from normal subjects (6, 33). Furthermore, the contractility of human bronchial smooth muscle cells from non-atopic subjects can be enhanced by incubation *in vitro* with human serum from atopic individuals containing immunoglobulin E (IgE) or by passive sensitization using human serum containing IgE (33). Newer methodologies in deriving ASM cell lines from subjects with asthma compared with age- and sex-matched controls have resulted in similar findings: bronchial smooth muscle cell lines derived from subjects with asthma exhibit enhanced shortening compared with cell lines from subjects without asthma (30, 41). ASM cells from patients with asthma embedded in collagen gels exhibited greater contraction in response to histamine than cells from normal subjects (31). All of these studies point to

phenotypic differences in the ASM cells from asthmatics compared with normals that result in enhanced contractility.

Investigators have shown that asthmatic-derived ASM has an amplified expression of CD38 (24). CD38 is a cell surface hydrolase and cyclase whose activation generates cADPR, a putative activator of the ryanodine receptor. Accordingly, increases in cADPR enhance agonist-induced ASM calcium mobilization (14). Importantly, CD38 expression is also induced by IL-13 and TNF, suggesting that CD38 may also play an important role in mediating airway hyperresponsiveness (24, 40).

These findings in human asthmatic ASM cells and tissues are supported by parallel findings in studies of ASM cells derived from animal models of airway hyperreactivity and asthma. The enhanced contractility and shortening *in vitro* of ASM isolated from animals with intrinsic airway hyperreactivity is well documented: ASM from the hyperresponsive Fisher rats is more reactive *in vitro* than that of Lewis rats (42); ASM from the hyperreactive Basenji greyhound dogs exhibits enhanced sensitivity to contractile agents compared with that of less reactive dogs (15); ASM from hyperreactive mouse strains exhibits enhanced contractility *in vitro* (5). Differences in ASM responsiveness also exist in airways isolated from animal models of allergen-induced airway hyperreactivity, indicating that the airway hyperresponsiveness observed in these animals can be attributed directly to the effects of sensitization on the excitation-contraction coupling in the ASM (11, 12, 32).

Inflammatory mediators present in the bronchoalveolar lavage of subjects with asthma (IL-4, IL-13, and TNF) also increase *in vitro* responsiveness of ASM to bronchoconstricting agents and decrease the relaxation of ASM to relaxing agents (11, 16, 17, 19, 26, 37). Although the precise mechanisms by which cytokines enhance agonist-induced excitation-contraction coupling remain unclear, a variety of calcium signaling pathways have been impugned (40). Furthermore, increases in shortening velocity also alter the responses of ASM to mechanical stretch that has been described as dysfunctional in asthma (1). Collectively, overwhelming evidence suggests that ASM derived from subjects with asthma manifest an enhanced excitation-contraction coupling response to bronchoconstricting agents. It is likely that this enhanced bronchoconstricting response coupled with an insensitivity to bronchodilators directly regulate airway hyperresponsiveness in asthma.

In addition to alterations in calcium mobilization pathways and contractile protein responses to agonists, ASM derived from asthma subjects also manifests a hyperproliferative phenotype and chemokine/cytokine secretion profiles that play important roles in mediating airway hyperresponsiveness. ASM cells derived from subjects with asthma compared with non-asthmatic subjects manifest greater cell proliferation at baseline and in response to mitogens. Importantly, the hyperproliferative ASM is less responsive to steroids and cAMP-mobilizing agents that are effective in inhibiting mitogen-induced ASM proliferation in cells derived from non-asthma subjects (13, 23, 43, 45). Because increases in ASM mass are one of the most commonly reported findings in the histopa-

thology of asthma and because increased ASM mass attenuates luminal diameter, it is likely that hypertrophy and hyperplasia of ASM may enhance the effects of agonist-induced ASM shortening that could prominently amplify airway hyperresponsiveness in asthma.

In addition to ASM shortening and proliferation, compelling evidence also suggests that ASM may serve immunomodulatory roles by secreting chemokines and cytokines. Numerous studies have suggested that in ASM, either in vivo using immunocytochemical staining or in ASM cell lines derived from subjects with asthma, there is enhanced chemokine/cytokine secretion compared with that from non-asthmatic subjects. Mast cell-ASM interactions were recently described and seemingly promote mast cell degranulation. These latter in vitro findings are in agreement with ex vivo ultrastructural analysis of ASM derived from asthmatic subjects using electron microscopy (4). Furthermore, the number of mast cells within ASM in vivo is positively correlated with the degree of airway hyperresponsiveness (8, 38) and with the intensity of α -smooth muscle actin staining (44). ASM cells also promote mast cell survival and proliferation through a mechanism involving a cooperative interaction between ASM membrane-bound SCF, soluble IL-6, and mast cell CADM1 (20). In addition to the unique interaction of ASM and mast cells in asthma, ASM-T cell interactions in asthma have also been described (4, 34). Two other ASM cell surface molecules, CD40 (9, 18, 25, 29) and OX40 ligand (10, 25, 39), are both expressed in ASM derived from asthmatic subjects and non-asthmatic subjects can also promote asthma cell-T cell adherence. The physiological relevance of these findings suggests that T cells can directly alter ASM contractile phenotypes by enhancing agonist-induced ASM shortening and reducing relaxation to isoproterenol (18). Moreover, T cells may induce ASM remodeling and more precisely ASM hyperplasia (28, 34). Indeed, in a rodent model of allergen-induced airway hyperresponsiveness, adoptively transferred CD40 T cells from OVA-sensitized rats induced an increase in ASM mass that was related to an increased ASM proliferation and decreased apoptosis ex vivo (35). In addition to the secretion of chemokines and cytokines and the interactions of immunocytes with ASM cells, ASM robustly secretes extracellular matrix (ECM) that can contribute to increasing ASM mass. The fractional area of the matrix is increased in ASM mass in cases of fatal asthma (3). ECM provides a scaffold to support the cells and serves as a reservoir for growth factors and matrix metalloproteinases (MMPs). The serum derived from subjects with asthma enhance ECM secretion by ASM (21), and the profile of matrix proteins secreted by ASM derived from subjects with asthma is markedly different than that secreted by ASM from non-asthmatic subjects (22, 27). The importance of these alterations in matrix proteins relates to the functional properties of ASM. Fibulin 1-C, for example, plays a role in vitro in enhanced proliferation of ASM derived from subjects with asthma. The components of the matrix may also modulate responses to pharmacotherapy, conferring insensitivity to glucocorticoids and β_2 -adrenergic agonists (7, 36). In studies of subjects who succumb to fatal asthma, a loss of fibrin and fibronectin is increased, and expression of MMPs, specifically MMP9 and MMP12, is dramatically different than that seen in non-asthmatic subjects (2).

Overall, compelling evidence suggests that ASM is dramatically different in subjects with asthma compared with non-asthmatic subjects, most notably excitation-contraction coupling, growth, and synthetic responses, all of which contribute to ASM function, likely regulate ASM hyperresponsiveness in asthma. Given this evidence, ASM, the pivotal cell regulating bronchomotor tone, is also the primary cell mediating airway hyperresponsiveness in asthma.

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COUNTERPOINT: ALTERATIONS IN AIRWAY SMOOTH MUSCLE PHENOTYPE DO NOT CAUSE AIRWAY HYPERRESPONSIVENESS IN ASTHMA

We have been assigned the task of showing that “alterations in airway smooth muscle phenotype DO NOT cause the airways hyperresponsiveness of asthma.” In the following paragraphs we will briefly review the overwhelming lack of evidence that any fundamental change in the intrinsic properties of