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Airway Smooth Muscle in Asthma: Just a Target for Bronchodilation?

Judith L. Black[1,2,*], **Reynold A. Panettieri Jr.**[3,*], **Audreesh Banerjee**[3,*], and **Patrick Berger**[4,5,6,*]

[1]University of Sydney, Pharmacology, Sydney, New South Wales, Australia

[2]Woolcock Institute of Medical Research, Sydney, New South Wales, Australia

[3]University of Pennsylvania Perelman School of Medicine, Department of Medicine, Pulmonary, Allergy and Critical Care Division, Airways Biology Initiative, Philadelphia, PA, USA

[4]Univ. Bordeaux, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, CIC 0005, F-33000 Bordeaux, France

[5]INSERM, U1045, CIC 0005, F-33000 Bordeaux, France

[6]CHU de Bordeaux, Service d'Exploration Fonctionnelle Respiratoire, CIC 0005, F-33604 Pessac, France

Synopsis

Airway smooth muscle (ASM) has long been recognized as the main cell type responsible for bronchial hyperresponsiveness. It has thus been considered as a target for bronchodilation. In asthma however, there is a complex relationship between ASM and inflammatory cells such as mast cells and T lymphocytes. Moreover, the increased ASM mass in the asthmatic airways is one of the key features of airway remodeling. This article aims to review the main concepts about the three possible roles of ASM in asthma including (i) contractile tone, (ii) inflammatory response and (iii) remodeling.

Keywords

Bronchodilators; Hyperresponsiveness; Inflammation; Remodeling; Smooth muscle

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Corresponding author Pr Patrick Berger, Centre de Recherche Cardio-thoracique de Bordeaux, INSERM U1045., Equipe: Remodelage bronchique, Université Bordeaux Segalen., 146 rue Léo Saignat., F33076 Bordeaux Cedex, France, Tel +33 5 57 57 16 94 Fax +33 5 57 57 16 95, patrick.berger@u-bordeaux2.fr.
[*]Authors contributed equally to this manuscript.

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Introduction

Asthma is a chronic disease, characterized by the association of bronchial hyperresponsiveness (BHR), inflammation, and remodeling (1). Airway smooth muscle (ASM) has long been recognized as the main cell type responsible for bronchial contraction and BHR (2). It has thus been logically considered as the key target for bronchodilation. Regarding asthmatic inflammation, a high number of eosinophils infiltrate both bronchial epithelium and submucosa (3), but there is apparently no eosinophil infiltration of the ASM layer (4). However, other inflammatory cells such as mast cells (4) or T lymphocytes (5) have been shown to infiltrate the ASM suggesting a complex relationship between ASM cells and inflammatory cells and even a possible role for ASM to organize inflammation. Asthmatic bronchial remodeling is characterized by various structural changes including abnormal epithelium, sub-epithelial membrane thickening, alteration of the extracellular matrix (ECM) deposition, neoangiogenesis, mucus gland hypertrophy and increased ASM mass (6). Recent evidence suggests that the increased ASM mass is the key feature of bronchial remodeling in asthma since, on the one hand, it is associated with severe asthma phenotype (7), and, on the other hand, it is correlated with a decrease in lung function (5, 8). This article reviews the main concepts about the three possible roles of ASM in asthma including (i) contractile tone, (ii) inflammatory response and (iii) remodelling.

Airway smooth muscle shortening and relaxation

In normal airways, ASM cell contraction regulates airway caliber and bronchomotor tone. Using isolated bronchial preparations, studies have compared the isotonic length and/or force generation of tissues derived from asthmatics and non-asthmatics. Interestingly, not all studies demonstrated an increased force generation in asthmatic tissues when compared to control tissues (9). These conflicting data likely can be attributed to differences in the experimental and methodological approaches used (10). Several parameters, such as the degree of tissue elastance, smooth muscle mass, and knowledge of the optimal length, were found to be important factors when evaluating the force generating capacity of ASM preparations derived from asthmatics (9, 10).

Although conflicting data exist in studies comparing smooth muscle responsiveness between normal and asthmatic patients, a number of studies report that passive sensitization of human ASM with asthmatic serum induces a non-specific increase in smooth muscle responsiveness (11–14), demonstrating the existence of mediators in the serum of asthmatic patients that promote airway responsiveness. While the precise nature of these mediators remains incompletely defined, evidence suggests that TNF-α, IL-13 or IL-1β can induce BHR in both humans and animals. Cytokines also can "prime" ASM to become hyperresponsive to contractile agonists *in vitro*, supporting the concept that cytokines modulate agonist-induced ASM contractile function. Other pro-inflammatory mediators, such as lysophosphatidic acid, a bioactive lipid released from activated platelets, phospholipase A_2 and leukotriene C4, also enhance ASM responsiveness *in vitro* to contractile agonists such as acetylcholine, methacholine and serotonin. Together, these studies suggest that pro-inflammatory mediators induce BHR by enhancing ASM contraction and/or altering ASM relaxation (see Figure 1). Understanding the mechanisms by which inflammatory mediators modulate ASM contractile reactivity may offer new insight into the molecular mechanisms that modulate BHR in asthma (reviewed in (15)).

The level of intracellular calcium regulates, in part, ASM shortening. Activation of an ASM cell by an agonist induces a rapid rise in $[Ca^{2+}]_i$, associated with the release of intracellular calcium stores, to a peak level roughly tenfold higher than the resting level (100 nM to greater than 1 μM with maximum agonist stimulation). Following this peak, calcium levels

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fall but remain elevated provided that the excitatory stimulus remains present. The elevation in $[Ca^{2+}]_i$ activates the calcium/calmodulin-sensitive myosin light chain kinase (MLCK), leading to phosphorylation of the regulatory myosin light chain (MLC_{20}) at Serine 19. Phosphorylation of this residue by myosin ATPase activity initiates crossbridge cycling between myosin and actin. ATP binding, hydrolysis and ADP release continue as long as MLC_{20} is phosphorylated; dephosphorylation by the MLC phosphatase terminates crossbridge cycling and relaxes smooth muscle (reviewed in (16)).

Considering the central role of Ca^{2+} in regulating ASM contractile function, investigators postulate that alterations in Ca^{2+}-regulatory mechanisms likely impair ASM contractility. Studies using cultured human tracheal or bronchial smooth muscle cells, as *in vitro* models of ASM responsiveness, convincingly demonstrated that Gq-protein coupled receptors (GPCR)-associated signaling in ASM can be modulated by a variety of inflammatory stimuli. Cytokines, such as TNF-α, augment agonist-induced ASM contractility by enhancing, in a non-specific manner, agonist-evoked Ca^{2+} transients (to bradykinin, carbachol) (15). The hypothesis that changes in GPCR-associated Ca^{2+} signaling represent an important mechanism underlying the development of BHR has also been supported by other studies. Tao and colleagues showed that ASM cells derived from hyperresponsive inbred rats have an augmented bradykinin-induced Ca^{2+} response when compared to ASM cells derived from normoresponsive rats (17). Deshpande and colleagues demonstrated that in addition to TNF-α, other cytokines including IL-1β and, in to a lesser degree, IFNγ augments Ca^{2+} responses induced by carbachol, bradykinin and thrombin (18). In a similar manner, IL-13, a Th2 type important mediator in allergic asthma (19), also non-specifically increased Ca^{2+} responses to agonists (20–23). Microarray technology used to study the modulation of gene expression of ASM by IL-13 revealed a diversity of potential molecular mechanisms influencing ASM responsiveness, including changes in cytoskeletal proteins, receptors or calcium regulators (24). Together, these data show that “pro-asthmatic” cytokines, in a non-specific manner, enhance GPCR-associated Ca^{2+} responses in ASM, a mechanism likely to affect ASM contractility.

Reports in C3H/HeJ, Balb/C and A/J mice revealed that differences in ASM contractility among species may not require changes in GPCR agonist-induced Ca^{2+} responses but rather involve changes in the Ca^{2+} sensitivity of the contractile apparatus (25). A possible mechanism involves the small monomeric G protein Rho that can augment ASM contractility by increasing levels of MLC phosphorylation via the Rho-activated kinase (ROCK) dependent suppression of MLC phosphatase (26, 27). Both RhoA and ROCK are activated by a variety of stimuli associated with the development of BHR including cytokines (28–31), sphingolipids (32–34), mechanical stress (35) and isoprostane (36). The RhoA/Rho kinase pathway regulates the expression of serum response factor-dependent smooth muscle specific genes in canine ASM cells (37), a mechanism that identifies the importance of the Rho-kinase pathway in maintaining a contractile phenotype recently described in bovine ASM tissues (38). Rho pathways modulate diverse cellular responses in ASM cells including the regulation of Ca^{2+} influx (39) and cell proliferation (40). Possibly, abnormal RhoA activity and/or expression will dramatically alter ASM contractility not only via the Ca^{2+} sensitization but also through the increased expression of Rho-dependent contractile proteins. A report using the Y-27632 inhibitor confirmed that the non-specific BHR as well as the specific allergen responsiveness induced by passive sensitization requires the activation of Rho-kinase (41).

Changes in ASM contractile properties play an important role in the development of BHR associated with chronic airway diseases such as asthma. *In vitro* studies support the concept that a variety of “pro-asthmatic” signals such as physical (repeated stretch) or chemical exposures (cytokines) drastically augment ASM contractile force by altering multiple key

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pathways: i) via the aberrant activation of contractile and/or impaired function of relaxant receptors (desensitization), ii) the alteration of Ca^{2+} regulatory signaling molecules (CD38, SERCA, Ca^{2+} channels), and iii) the activity of elements of the contractile apparatus through Rho-dependent pathways. Defining the inflammatory signals (factors and associated mechanisms) involved in the regulation of ASM responsiveness may represent a potential new target for the treatment of BHR. Pro-inflammatory mediators can modulate the density of contractile agonist receptors on ASM cells. TNF-α induced a dramatic decrease in muscarinic receptor density. In contrast, expression of the bradykinin B_2 receptor was rapidly increased in ASM exposed to IL-1β or TNF-α by a prostanoid-dependent regulation of gene transcription and by the activation of the Ras/Raf/MEK pathway. Surprisingly, the β_2-agonist fenoterol or the steroid methylprednisolone also increased expression of the histamine H_1 and bradykinin B_2 receptors, an effect that involved both increased gene expression and mRNA stability. This increase in H_1 receptor expression was associated with an increase in the contractile response to histamine. Whether β_2-agonists and steroids induce such effects *in vivo* remains unclear. These studies, however, suggest that current asthma therapy may also modulate BHR by altering contractile agonist receptor expression in ASM (reviewed in (15)).

In human ASM cells, contractile agonists bind GPCR and activate phospholipase C. The subsequent hydrolysis of phosphatidylinositol 4,5 bisphosphate into inositol trisphosphate and diacylglycerol ultimately results in an increase in $[Ca^{2+}]_i$ (reviewed in (16)). Since most of the inflammatory agents do not evoke either a calcium response or phosphoinositide hydrolysis in human ASM, modulation of agonist-induced increases in $[Ca^{2+}]_i$ by extracellular stimuli may be due to the modulation of downstream GPCR signaling. TNF-α increased the amount, as well as the activity, of G-proteins in several cell types including ASM (42, 43). The finding that TNF-α potentiated calcium mobilization in response to NaF (44), an agent that bypasses membrane receptors and directly activates G-proteins (45, 46), supports the notion that TNF-α may act directly at the level of G-proteins.

Studies now show that bradykinin-evoked phosphoinositide accumulation in human ASM is significantly enhanced by various cytokines such as TNF-α and IL-1β (44, 47–49). The effect of cytokines on agonist-evoked calcium responses seems to be stimulus-specific, however, since pretreatment of ASM cells with IL-1β diminished phosphoinositide metabolism induced by histamine (50). In addition to their effect on calcium signaling, cytokines may also modulate β_2-adrenergic function. TNF-α as well as IL-1β also suppress isoproterenol-stimulated activation of adenylyl cyclase (51–53). A recent report showed that IL-13 is also able to impair ASM responsiveness to β_2-adrenergic stimuli (54) showing that cytokines may promote BHR by impairing β_2-adrenergic responsiveness in ASM cells.

Pro-inflammatory cytokines affect ASM contractility on many levels. Alterations in calcium homeostasis and sensitivity, as well as contractile agonist receptor expression and signal transduction pathways, have profound effects on airway hyperreactivity. The ability of anti-inflammatory therapies, such as corticosteroids, to modulate these effects are discussed later in this review (also reviewed in (15, 55)).

To date, the most effective therapeutic approaches in asthma are corticosteroids and β_2-adrenergic receptor agonists, which abrogate airway inflammation and reverse bronchoconstriction respectively. Given the evidence that ASM cells secrete and express immunomodulatory proteins, investigators are now studying the cellular and molecular processes that regulate ASM synthetic function and examining the role of dexamethasone and β-agonists in modulating cytokine-induced synthetic responses and bronchodilation.

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In asthma, β-agonists such as isoproterenol, albuterol, salmeterol and formoterol are therapeutic agents that promote bronchodilation by stimulating receptors coupled to Gs, and that, in turn, activate adenylyl cyclase, increase $[cAMP]_i$ and stimulate cAMP-dependent protein kinase (A-kinase) in ASM. In a similar manner, PGE_2, which is produced in large quantities at sites of inflammation, also increases $[cAMP]_i$ in human ASM cells and is a potent and effective bronchodilator (56). Evidence suggests that $[cAMP]_i$ mobilizing agents in ASM cells also modulate cytokine-induced synthetic function. In TNF-α-stimulated ASM cells, both eotaxin and RANTES expression are effectively inhibited by isoproterenol, PGE_2, dibutyl-$[cAMP]_i$, or the phosphodiesterase inhibitors, rolipram and cilomast (57–59). TNF-α-induced IL-8 secretion was also inhibited by the combination of $[cAMP]_i$ mobilizing agents and corticosteroids (60). Similarly, sphingosine-1-phosphate, which activates a Gs protein coupled receptor and increases $[cAMP]_i$, abrogated TNF-α-induced RANTES secretion in ASM cells (61).

In contrast to the effects of $[cAMP]_i$ on chemokine secretion, pharmacological agents that increase $[cAMP]_i$ also markedly stimulate secretion of IL-6 in human ASM cells (57). This appears to be due to effects on basal IL-6 promoter activity (62). Whether the secreted IL-6 modulates ASM cell function in an autocrine manner or alters leukocyte function in the submucosa remains unknown. Since studies show that overexpression of IL-6 decreases acetylcholine responsiveness in transgenic mice (63), the role of IL-6 in asthma may be that of an anti-inflammatory signal. More recently, investigators reported that cAMP limits secretion of GM-CSF by ASM cells. Cyclo-oxygenase inhibitors reduce PGE_2 and enhance cytokine-induced secretion of GM-CSF (64, 65), while PDE type IV inhibitors reduce GM-CSF secretion *in vitro* and antigen-induced BHR in an animal model (64, 66). Taken together, current evidence suggests that some but not all pro-inflammatory functions in ASM cells are inhibited by $[cAMP]_i$ mobilizing agents.

Conflicting reports exist, however, concerning effects of increased $[cAMP]_i$ on lymphocyte adhesion and migration through cytokine-activated endothelial cells (67, 68). The controversy regarding the role of $[cAMP]_i$ in modulating cell adhesion likely reflects differences in the cytokines used to stimulate endothelial cells, or the temporal differences in the addition of the agonists used to increase $[cAMP]_i$. Far less is known concerning $[cAMP]_i$ effects on smooth muscle-leukocyte adhesion. In human ASM cells, activation of $[cAMP]_i$-dependent pathways inhibited, in part, both TNF-α-mediated induction of ICAM-1 and VCAM-1 expression and adhesion of activated T cells to ASM cells. Interestingly, the basal expression of ICAM-1 and VCAM-1, as well as the binding of activated T cells to unstimulated ASM, was resistant to increases in $[cAMP]_i$ (69). Together these studies suggest that cytokine-induced expression of cell adhesion molecules and T cell adhesion to ASM cells are modulated by changes in $[cAMP]_i$.

Airway smooth muscle can also organize inflammation

There is plenty of evidence that, in asthma, a complex relationship involves ASM and inflammatory cells. Indeed, ASM produces a variety of chemotactic mediators and expresses different adhesion molecules (Table 1), which can participate to both the recruitment and the micro-localization of inflammatory cells within the ASM. Asthmatic ASM is infiltrated by both mast cells (4) and T lymphocytes (5) but apparently no eosinophil (4).

The mast cell infiltration of the ASM layer, also called mast cell myositis (70) appeared to be a specific feature of asthma, as compared to that of patients suffering from eosinophilic bronchitis and healthy subjects (4, 71). Interestingly, the ASM mast cell infiltration is observed in various asthma phenotypes, including eosinophilic and non eosinophilic asthma (72), severe and non severe asthma (71, 73–75), but also atopic and non atopic asthma, even

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if the number of mast cells were significantly higher in the ASM of atopic asthmatics (76). Moreover, asthma treatments including inhaled corticosteroids did not change mast cell myositis (71, 73). The mechanism of such a myositis has been firstly related with the production of mast cell chemotactic factors by the ASM itself, through an auto-activation loop (77). Indeed, upon activation, mast cells release tryptase and pro-inflammatory cytokines, such as TNF-α, which stimulate the production of TGF-β_1 and, to a lesser extent, SCF by ASM cells, which in turns, induce mast cell chemotaxis (77). Moreover, ASM promote mast cell chemotaxis through the secretion of a wide array of chemotactic factors, upon stimulation by Th1 (78), Th2 (78, 79) or pro-inflammatory cytokines (77, 80). ASM also produces functionally active CXCL10 (78), CXCL8 (79), CCL11 (79) and CX_3CL1 (80), even if for CX_3CL1, the additional presence of vasoactive intestinal peptide (VIP) is necessary (80). Taken together, mast cell migration is induced by the production of various mediators secreted by the ASM itself, which is closely related to the ASM inflammatory micro-environment.

Once present within the ASM bundle, mast cell can adhere to ASM. This adhesion has been initially reported to a cell - cell direct interaction involving an Ig superfamily member, *i.e.* CADM1 (cell adhesion molecule 1), previously known as TLSC-1 (tumor suppressor in lung cancer 1) (81, 82) (Figure 2). However, blocking CADM1 partially reduced the adhesion of mast cells to ASM, suggesting an alternative mechanism (81). Indeed, mast cell - ASM adherence also involved cell - extracellular matrix (ECM) - cell interaction through type I collagen, CD44 and CD51 (83) (Figure 2). This adhesion was improved under inflammatory conditions or using asthmatic ASM cells (83). These latter *in vitro* findings are in agreement with *ex vivo* ultrastructural analysis of asthmatic ASM using electron microscopy (84). Indeed, such analysis did not demonstrate any direct cell-cell contact between ASM and mast cells, but only close contacts without tight junction (84). The majority of mast cells infiltrating the asthmatic ASM bundles are typically of the MC_{TC} phenotype, containing both tryptase and chymase (4, 85, 86). Interestingly, these mast cells infiltrate the ASM of both large and small airways, and exhibit marked features of chronic ongoing activation (74, 76). Such findings were also confirmed by ultrastructural analysis of asthmatic ASM using electron microscopy (84). However, little is known about the mechanisms by which mast cell activation may occur within the ASM layer (87). Mast cell degranulation may result from IgE-dependent activation, especially in atopic patients (76). However, IgE-independent mechanisms have also been evoked, following mast cell - ASM interaction through the complement C3a or SCF (82, 88–90), for instance, or, following bacterial or viral infection through Toll-like receptors (87).

Taking into account the micro-localization of mast cells within the asthmatic ASM layer, the adherence of mast cells to the ASM, and, the features of mast cell activation within the ASM, it may be suggested a close functional relationship between these two cell types. On the one hand, mast cells are likely to alter functional and phenotypic properties of ASM cells. Indeed, mast cell-derived mediators may contribute to BHR (see part 1) and ASM remodeling (see part 3) (6). For instance, the major mast cell product tryptase induces ASM calcium increase (91) and non specific BHR to histamine *in vitro* (92) and *in vivo* (93). Tryptase also increases the production TGF-β1 by ASM cell (77), which promotes the differentiation of ASM cell towards a more contractile phenotype, characterized by both an increased expression of α-smooth muscle actin and an enhanced ASM contractility (88). The number of mast cells within the ASM layer is positively correlated with the degree of BHR (4, 71), and with the intensity of α-smooth muscle actin staining (88). Furthermore, mast cell myositis may also promote ASM remodeling. Indeed, mast cell-derived tryptase has been shown to stimulate ASM proliferation (94). Moreover, mast cell – derived CCL19 mediate ASM migration through the activation of ASM CCR7 (95). However, neither ASM proliferation (89, 96) nor ASM survival appeared to be modulated by co-cultured mast cells

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(89). In addition, CCL11/CCR3 mediated ASM cell migration was inhibited by mast cells (97). Furthermore, no correlation was found between the number of mast cells within the ASM and ASM mass, supporting the modest role of mast cells in ASM remodeling (76). On the other hand, ASM cells can alter mast cell functional and phenotypic properties. Indeed, ASM cells can 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 (82). ASM cell-derived ECM proteins may also promote mast cells differentiation towards a fibroblastoid phenotype, characterized by the expression of fibroblast markers and fibroblast-like morphology. This feature seems to be specific of mast cells within the ASM layer, since fibroblast markers were not expressed by mast cells within the submucosa (75).

Regarding T cell infiltration within the asthmatic ASM layer, only few studies have been performed. $CD4^+$ T cell micro-localization was initially reported in an experimental asthma model within the ASM layer of OVA-sensitized rat as compared to non sensitized animals (98). These findings have been further confirmed in human asthmatics (5, 84) and are related to asthma severity (5). As compared to mast cell infiltration, the number of T cells seems to be lower within the ASM layer (4, 84). ASM cells are able to produce appropriate chemotactic factors for T cells, including CCL5 (RANTES) or CXCL10 (IP-10) (78). However, direct chemotactic properties of ASM to T cells remain to be demonstrated.

Close contacts between ASM and T cells have been shown in asthma (5, 84), suggesting cell - cell adherence between these two cell types. Lazaar and coworkers firstly demonstrated that activated T cells can adhere *in vitro* to resting ASM cells from non asthmatic patients and that such an adhesion was enhanced when ASM cells were primed with pro-inflammatory cytokines such as TNF-α (99). These findings were further confirmed independently (100). This adhesion involves on the one hand, CD44, ICAM-1 (Intercellular cell adhesion molecule 1), VCAM-1 (Vascular cell adhesion molecule 1) expressed by ASM cells, and, on the other hand, CD44, LFA-1 (Lymphocyte function-associated antigen 1) and VLA-4 (Very late antigen 4) expressed by T cells (99). More recently, non asthmatic ASM cells, pulsed to the superantigen staphylococcal enterotoxin A (SEA), have been shown to adhere to T cells by presenting the SEA *via* their MHC class II (101). Although ASM cells express MHC class II constitutively and under stimulation (100, 102), these cells are not classically considered as an antigen presenting cell. Consequently, these findings support an emerging role of ASM cell as an immunomodulatory cell. However, except for VCAM1, which forms clusters in the asthmatic ASM *ex vivo* suggesting VCAM1-mediated intercellular signaling, the role of the above molecules in adherence between T cell and ASM cell from asthmatic patients has not yet been considered. Two other ASM cell-surface molecules, CD40 (100, 103–105) and OX40 ligand (105–107), both expressed in asthmatic and non asthmatic ASM cells, have also been suggested in promoting ASM cell – T cell adherence. These co-stimulatory cell-surface molecules, members of the TNF superfamily, respectively bind to CD154 and OX40 on activated T cells (108, 109). However, their role in the adherence of T cells to asthmatic ASM remains to be investigated. By contrast, a possible role for mast cell chymase has been suggested since this mast cell protease is able to inhibit T cell adhesion to non asthmatic ASM cells *in vitro* (110).

Close interaction between T cells and ASM cells can stimulate a cross-talk between these two cell types, but little is known about the functional consequences of such interaction. On the one hand, T cells may alter functional properties of ASM cells. Firstly, T cells may alter ASM contractile phenotype, enhancing ASM contractility to acetylcholine and reducing its relaxation to isoproterenol in isolated rabbit bronchi (100). Moreover, T cells may also induce ASM remodeling, and more precisely ASM hyperplasia (5, 98, 99). Indeed, in an experimental rat asthma model, adoptively transferred $CD4^+$ T cells from OVA-sensitized rats induced an increase in ASM mass, which was both related with an increased ASM

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proliferation and decreased apoptosis *ex vivo* (98). Such findings were confirmed *in vitro* only upon direct $CD4^+$ T cells – ASM cells contact, highlighting the need for close cellular interaction between these two cell types (98). These observations are in agreement with a previous study demonstrating *in vitro* a role for T cell in ASM DNA synthesis and proliferation in ASM cells from non asthmatic patients (99). The role of T cells in driving ASM remodeling was later confirmed in human asthmatics (5). Moreover, the number of T cells infiltrating the asthmatic ASM correlated with ASM mass (5). Collectively, these findings suggest an emerging role of T cells in both ASM hyperresponsiveness and remodeling. On the other hand, ASM cells may also alter functional properties of T cells. Indeed, direct contact between $CD4^+$ T cells and ASM cells also enhances T cell survival, thus possibly contributing to the perpetuation of bronchial inflammation (98). Cultured human non asthmatic ASM cells are able to present superantigens *via* their MHC class II molecules to resting $CD4^+$ T cells (101), which induces $CD4^+$ T cells activation, adherence between these cells and finally the release of IL-13, that, in turns, allow increasing the contractile response to acetylcholine of isolated rabbit bronchi (101).

Airway smooth muscle remodeling

The fact that the increased ASM mass in the asthmatic airway is one of the key features of the structural changes which constitute airway remodeling is well recognized (111). Moreover this increase has been attributed with a pivotal role in determining one of the key functional characteristics of the asthmatic airway namely airway hyperresponsiveness (112). The increased muscle mass is likely to be the most important abnormality responsible for the exaggerated response to bronchoconstricting stimuli in asthma, resulting in development of greater narrowing of the lumen. More recently there has been much investigation into the mechanisms underlying this increase. Whether hyperplasia, hypertrophy, or their combination is the major contributing process remains unclear, and it is possible that the changes in the muscle are not homogeneous throughout the airway. Ebina et al (113) described two patterns in lungs harvested post mortem – type I in which hyperplasia was present in the walls of large bronchi with no hypertrophy and type II in which hypertrophy was present in the whole airway and mild hyperplasia in the larger airways. Additional theories as to the reason for the increase in muscle bulk relate to an increase in ECM proteins deposited in and around the muscle bundles (see below) and migration of mesenchymal precursors from the peripheral blood into the lung (114).

Although the majority of research has focused recently on the mechanisms underlying hyperplasia, some interesting observations have been reported regarding hypertrophy and, specifically, that induced by stretch. This is important because bronchoconstriction itself constitutes a form of stretch or strain (115) and this is highly relevant to the asthmatic airway. Interestingly, the effect of mechanical stretch is to induce hypertrophy of human ASM and this appears to be mediated by the action of a microRNA (miRNA) specifically miR-26a (116). Stretch and/or overexpression of miR-26a induces human ASM hypertrophy via an effect on glycogen synthase kinase -3β. In these experiments, stretch also induced hyperplasia but the predominant effect was hypertrophy and miR26a did not affect proliferation.

A lot more attention has been focused on the underlying causes of hyperplasia which, in some studies, has been reported to be the sole pathology underlying the increase in muscle mass (117). This has been attributed to an upregulation of proproliferative pathways or, alternatively, a lack of endogenous braking mechanisms (Table 2). In 2001 (118), it was noted that ASM cells obtained from patients with asthma proliferated at a greater rate than those obtained from people without asthma and this finding was confirmed and extended by Trian et al (119) although this has not always been a consistent finding (89, 120). In the

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latter study, the increased growth was attributed to mitochondrial biogenesis and to dysfunctional calcium regulation (119). Further studies on calcium homeostasis have demonstrated that the expression and function of sarcoendoplasmic reticulum calcium ATPases, which play a role in the extrusion of calcium from the ASM cell, are downregulated (121, 122). With respect to hyperproliferative pathways, almost every signal transduction protein has been associated with exaggerated proliferation, including, but not limited to the MAP kinases ERK (123) and p38 (124), as well as PI3 kinases (125). Studies conducted using intact tissue, as opposed to cells from patients with asthma, have revealed increased immunohistochemical detection of a marker of proliferation – proliferative cell nuclear antigen, indicating that evidence for increased proliferation of ASM is not merely a phenomenon of cell culture (5, 126).

It is possible that internal mechanisms for limiting proliferation could be dysfunctional (Table 2). Levels of endogenous Prostaglandin E_2, which is an inhibitor of mitogenesis (127), are decreased in cultures of asthma derived ASM, and this was also found to be the case for protein levels of the transcription factor C/EBPα- an inhibitor of proliferation (128). In addition, evidence that vitamin D inhibits ASM cell proliferation may suggest that low levels *in vivo* could contribute to unopposed growth (129).

It was originally thought that the structural changes in asthmatic airways that constitute airway remodeling developed as a result of a persistent inflammatory stimulus. This has been challenged by some recent reports regarding the mechanisms underlying remodeling. Bronchoconstriction induced by a non inflammatory stimulus such as methacholine, resulted in significant increases in parameters of remodeling to approximately the same extent as an allergen challenge (130). These changes were assessed by means of biopsies, and, although there was no actual measurement of ASM, the number of proliferating cells in the lamina propria was increased in each challenge. The exact nature of these cells was not explored but they could have been myocytes which had dedifferentiated into myofibroblasts and migrated into the submucosa in response to allergen challenge (131). Increases in sub basement membrane collagen and alterations in epithelial cell morphology – both hallmarks of airway remodeling- were a consistent feature observed in the study by Grainge (130) in response to both inflammatory (allergen) and non inflammatory (methacholine) challenges. Thus, it may be that the structural changes which constitute remodeling are not a consequence of chronic persistent inflammation, as was previously thought, but rather the two processes develop along separate parallel pathways- a hypothesis put forward by Martinez in 2007 (132).

Not only ASM cells per se but also their products – eg ECM proteins contribute to the area of the muscle bundle (133). The fractional area of the matrix is increased in the smooth muscle in cases of fatal asthma (134). The ECM provides a scaffold for support of the cells embedded in it. In addition, ECM proteins have profound biological effects on the smooth muscle – influencing proliferation, formation and release of growth factors, and matrix metalloproteinases (MMPs) which may cleave factors embedded in the matrix and release them to alter function. There is now accumulating evidence that remodeling in the asthmatic airway is associated with changes in the ECM proteins. When ASM cells are exposed to serum from asthmatic patients, increases in many ECM proteins are observed (135). Alterations in the ECM are a well recognized component of remodeling in the asthmatic airway, including enhanced deposition of collagens I, III and V, fibronectin, tenascin, hyaluronan, versican, laminin α2/β2, and perlecan (136–139), whereas decorin, collagen IV and elastin are decreased (140). ASM cells derived from patients with asthma produce a different profile of matrix proteins (141) including increased amounts of fibulin 1-C (142). The importance of these alterations in the matrix proteins lies in the fact that they can profoundly alter the properties of the ASM. Fibulin 1-C for example plays a role *in vitro* in the enhanced proliferation of asthmatic ASM. The components of the matrix may also

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modulate response to pharmacotherapy, conferring resistance to corticosteroids and β2 adrenoceptor agonists (143, 144). In intensive studies of tissue from asthma patients who have suffered a fatal attack, elastic fibers and fibronectin are increased and in this same cohort, changes in the MMPs were also noted, with increases in both MMP-9 and -12 detected immunohistochemically in the ASM of large airways (145). Whether these changes are consistent throughout the airways is unclear.

Conclusion

A better knowledge of the various roles of ASM in the pathophysiology of asthma is necessary to understand that ASM is not just a target for bronchodilation. Of course, changes in ASM contractile properties and/or impaired function of relaxant receptors play an important role in the development of BHR in asthma. Bronchodilators can also inhibit some but not all pro-inflammatory functions of ASM *in vitro*. Moreover, steroids are not able to reduce both mast cell myositis and increased proliferation of asthmatic ASM. This latter appears to be an important mechanism of ASM remodeling. Moreover, T cells may induce ASM hyperplasia and have been involved in BHR. All these findings confirm that the roles of ASM in the pathophysiology of asthma are complex and need further specific pharmaceutical developments.

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Key Points

1. A better knowledge of the various roles of ASM in the pathophysiology of asthma is necessary to understand that ASM is not just a target for bronchodilation.
2. Changes in ASM contractile properties and/or impaired function of relaxant receptors play an important role in the development of BHR in asthma.
3. The roles of ASM in the pathophysiology of asthma are complex and need further specific pharmaceutical developments.

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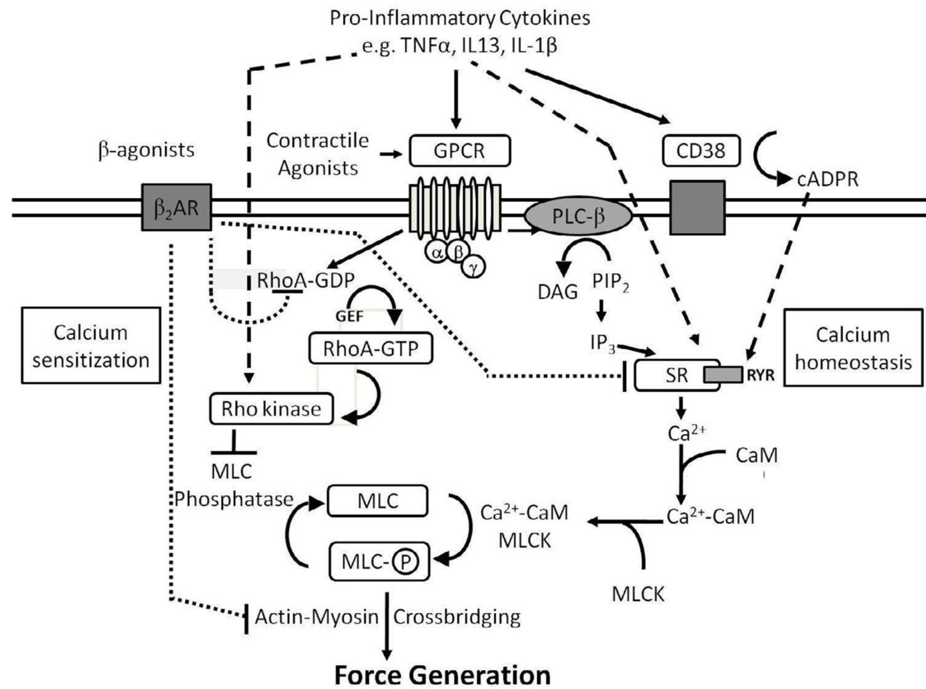

Figure 1. Excitation-contraction coupling in airway smooth muscle

Effects of pro-inflammatory cytokines and β_2-adrenergic receptor agonists on excitation-contraction coupling in ASM cells. Contractile agonists activate receptors that influence intracellular signaling, affecting calcium homeostasis and sensitization as well as the function and expression of GPCRs and CD38. Inflammatory cytokines bind to receptors and modulate calcium homeostasis by increasing expression of CD38 and increasing Ca^{2+} release from the sarcoplasmic reticulum. Inflammatory cytokines such as IL-13, IL-1β and TNF-α also increase Rho kinase activity to modulate the calcium sensitization pathways. β_2-adrenergic receptor agonists regulate calcium homeostasis and calcium sensitization by inhibiting RhoA activation, Ca^{2+} release from the sarcoplasmic reticulum, and actin-myosin crossbridging. cADPR, cyclic ADP ribose; CaM, calmodulin; DAG, diacylglycerol; GEF, guanine exchange factor; GPCR, G-protein–coupled receptor; IP_3, inositol tri-phosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; PIP_2, phophatylinositol 4,5 biphosphate; PLC, phospholipase C; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; β_2AR, β_2-adrenergic receptor.

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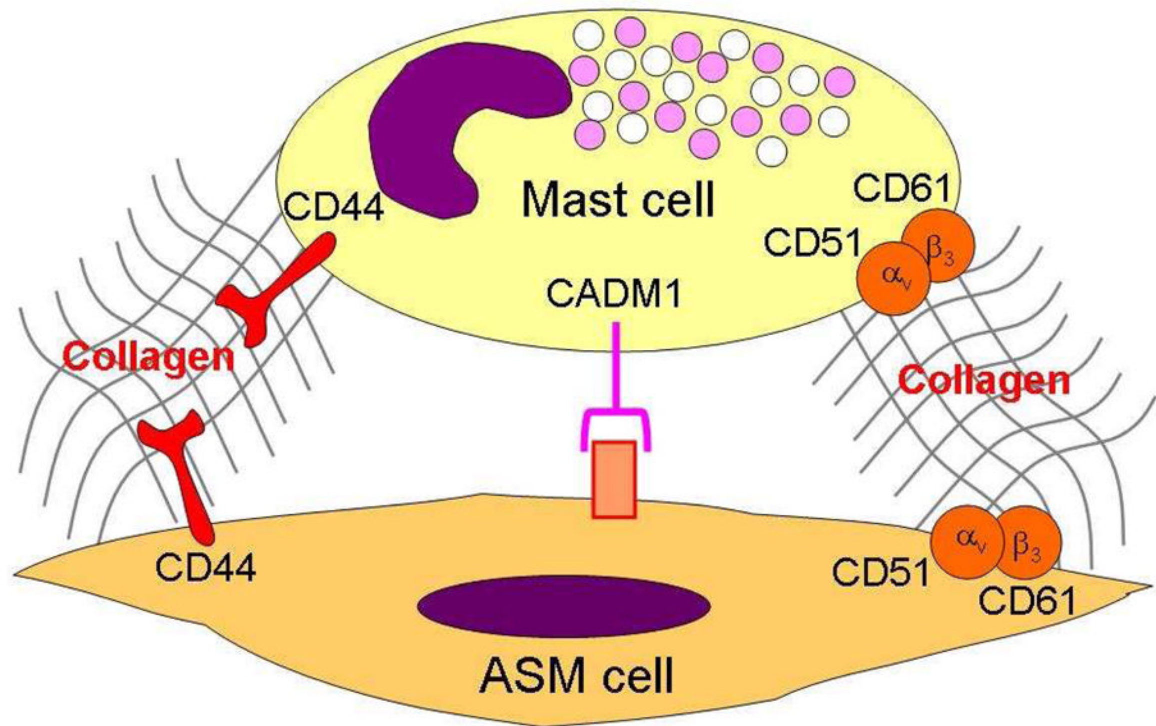

Figure 2. Airway smooth muscle - mast cell interaction

Airway smooth muscle (ASM) can adhere to mast cell through cell-cell direct interaction involving cell adhesion molecule 1 (CADM1) and through cell-extracellular matrix interaction involving type I collagen and both CD44 and CD51.

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Table 1

Airway smooth muscle production of chemotactic mediators and adhesion molecules

Factors	Spontaneous	After stimulation	References
Chemokines			
CCL2 (MCP-1)	−	+ (IL-1β, TNF-α)	(146, 147)
CCL5 (RANTES)	−	+ (IL-1β, TNF-α)	(147–149)
CCL11 (Eotaxin)	+	+ (IL-1β, TNF-α)	(78, 150, 151)
CCL19 (MIP-3β)	+		(95)
CXCL8 (IL-8)	−	+ (IL-1α, IL-1β, TNF-α)	(78, 146, 152)
CXCL9 (Mig)	−	+ (IFN-γ, IL-1β, TNF-α)	(78)
CXCL10 (IP-10)	−	+ (IFN-γ, IL-1β, TNF-α)	(78)
CXCL12 (SDF-1α)	−	+ (IFN-γ, IL-1β, TNF-α)	(78)
CX_3CL1 (Fractalkine)	−	+ (TNF-α)	(80)
Cytokines			
SCF	+		(153)
TGF-β1	+	+ (Angiotensin II)	(154)
IFN-γ	+	+ (AS)	(155)
GM-CSF	+	+ (AS, IL-1β, TNF-α)	(155–157)
L-2	+	+ (AS)	(155)
IL-5	+	+ (AS)	(155)
IL-6	−	+ (IL-1, TGF-β, TNF-α)	(149, 158)
IL-11	−	+ (IL-1, TGF-β, virus)	(158)
IL-12	+	+ (AS)	(155)
IL-33	+	+ (IFN-γ, TNF-α	(159)
Adhesion and co-stimulatory molecules			
CD11a	+		(100)
CD40	+	+ (TNF-α, IFN-γ)	(103)
CD40L	+		(103)
CD44	+		(99)
CD80, CD86	+		(100)
ICAM-1	+	+ (TNF-α, IL-1β, IL-5, AS)	(99, 160, 161)
VCAM-1	+	+ (TNF-α, IL-1β)	(99, 160)

Abbreviations: AS: asthmatic serum, GM-CSF: Granulocyte macrophage colony-stimulating factor, ICAM: intercellular adhesion molecule, IL: interleukin, IFN: interferon, SCF: stem cell factor, TGF: transforming growth factor, TNF: tumor necrosis factor, VCAM: vascular cell adhesion molecule.

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Table 2

Modulation of human airway smooth muscle proliferation[#]

Proproliferative	Antiproliferative
Growth factors	
EGF (162)	PGE_2 (127, 163)
IGF (164)	heparin (163)
PDGF (165)	interleukin-13 (166)
FGF-2 (167)	VIP (168)
Plasma/Inflammatory cell mediators	
β-hexosaminidases (169)	Glucocorticoids [^] (128)
Elastase (170)	interleukin-4 (167)
α-thrombin (171)	beta agonists (127)
Tryptase (94)	
Sphingosine-1 phosphate (61)	
Contractile agents	
Endothelin-1 [*] (162)	
Leukotriene D4 [*] (172)	
Cysteinyl Leukotriene (173)	
Histamine (168)	
Extracellular matrix proteins	
Fibronectin (174)	
Collagen-1 (174)	

Abbreviations: EGF: epidermal growth factor, IGF- insulin-like growth factor, PDGF: platelet derived growth factot, FGF-2: fibroblast growth factor-2,

[*] a co-mitogen,

[#] reviewed in Hirst *et al.*, (175). VIP: vasoactive intestinal peptide,

[^] nonasthmatic cells only