Airway structural components drive airway smooth muscle remodeling in asthma

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Chapter 2

Abstract

Chronic asthma is an inflammatory airways disease characterized by pathological changes in the airway smooth muscle bundle that contribute to airway obstruction and hyperresponsiveness. Remodeling of the airway smooth muscle is associated with an increased smooth muscle mass, involving components of cellular hypertrophy and hyperplasia, and changes in the phenotype of the muscle that facilitate proliferative, synthetic and contractile functions. These changes are considered major contributing factors to the pathophysiology of asthma, because of their role in exaggerated airway narrowing. The mechanisms that regulate changes in airway smooth muscle mass and phenotype are incompletely understood, but likely involve the regulatory role of mediators and growth factors secreted from inflammatory cells on airway smooth muscle cell proliferation and phenotype. An alternative hypothesis is that cellular and structural components that together constitute the airway wall, such as the airway epithelium, airway nerves, and the extracellular matrix, interact with the airway smooth muscle bundle to facilitate changes in smooth muscle phenotype and function that drive remodeling under inflammatory conditions. This review will discuss the mechanisms by which structural components of the airway wall communicate with the airway smooth muscle bundle to regulate remodeling and discuss these mechanisms in the context of the pathophysiology of asthma.

Introduction

Asthma is a chronic disease of the airways, which is characterized by persistent airway inflammation, reversible airways obstruction, airway remodeling and airway hyperresponsiveness (1). Airway hyperresponsiveness is defined by an exaggerated narrowing of the airways to a variety of chemical, physical and pharmacological stimuli (2). Acute, variable airway hyperresponsiveness has been considered to reflect increased airway smooth muscle contraction associated with airway inflammation and is related to asthma activity and severity, whereas chronic persistent airway hyperresponsiveness may reflect airway remodeling (3). Airway remodeling is characterized by changes in the structure of the airway wall, which include shedding of the epithelium, goblet cell hyperplasia, increased blood vessel number and area, increased and changed deposition of extracellular matrix (ECM) and increased airway smooth muscle (ASM) mass (4-6). Airway remodeling, notably the abnormalities in the ASM that encircles the airways and regulates lumen diameter, may contribute to the pathogenesis and pathophysiology of asthma.

Several studies have indicated that differences in contractile responses exist between ASM cells derived from asthmatics and cells derived from healthy subjects, which could at least in part explain hyperresponsiveness in asthma.
Thus, asthmatic ASM cells contract with greater velocity and maximum shortening capacity compared to healthy ASM (7). These changes may be explained by increases in the expression of smooth muscle myosin light chain kinase (sm-MLCK), transgelin (SM22) and myosin heavy chain (sm-MHC) as reported in asthmatic ASM cells and in asthmatic biopsies (7;8). Moreover, asthmatic ASM appears to express increased levels of the 7 amino acid insert SM-B isoform of sm-MHC, which shows a two-fold greater ATPase activity and shortening velocity compared to the SM-A isoform (8;9), although this latter finding is at odds with the study by Ma et al. who reported no expression of the SM-B isoform in smooth muscle obtained from asthmatic subjects (7). The increase in contractile protein expression may be of clinical relevance as expression levels of sm-MHC, sm-α-actin and desmin correlate with methacholine responsiveness in asthmatics (8;10). Studies using asthmatic and nonasthmatic ASM cultured in collagen gels also showed that maximal histamine-induced condensation of the gel was increased when cells derived from asthmatics were used (11). In addition, ASM relaxation may also be changed as relaxation of passively sensitized ASM is slower compared to controls (12).

Increased ASM mass is one of the most striking features of airway remodeling in asthma. Mathematical modeling studies on the impact of remodeling on airway narrowing indicated that increased ASM mass is likely to be the most important feature in increased airway narrowing in asthma, when assuming that the capacity of the ASM bundle to produce force is proportional to its mass (13;14). This idea is underscored by the fact that asthmatic patients in which the ASM layer has been reduced by bronchial-thermoplasty, show improved asthma control (15).

Several studies have addressed the underlying pathology causing the increased ASM mass in asthma. Ebina et al. (16) examined the ASM layer surrounding the airway lumen in fatal asthma and found two different asthmatic phenotypes, one showing an increased number of ASM cells (hyperplasia), the other showing an increased ASM cell size (hypertrophy). In subsequent studies, Woodruff et al. (17) found evidence for hyperplasia, but not hypertrophy in the ASM layer of mild to moderate asthmatics, whereas Benayoun et al. (18) found ASM hypertrophy, but not hyperplasia in patients with intermittent, mild-to-moderate and severe asthma. The latter group also found a clear correlation between disease severity and the degree of ASM thickening, consistent with a recent study showing that ASM thickening is more significant in fatal asthma as compared to non-fatal asthma (19). The relationship between age and duration of disease and ASM thickening is still subject of debate (18-21). Collectively, these findings suggest that increased ASM mass in asthma may reflect both cellular hyperplasia and hypertrophy, the degree of which depends primarily on asthma severity.

The increased ASM mass may be explained by intrinsic changes in the asthmatic ASM cell that facilitate their proliferative and secretory characteristics.
Asthmatic ASM produces more pro-inflammatory, pro-angiogenic and pro-
remodeling factors including eotaxin, vascular endothelial growth factor (VEGF)
and connective tissue growth factor (CTGF) (22-24), and less anti-mitogenic
factors like PGE\textsubscript{2} (25). Accordingly, asthmatic ASM cells in culture proliferate
faster compared to healthy controls (26), which is caused by changes in ECM
protein deposition (27), and by enhanced mitochondrial biogenesis and
mitochondrial activity that support increased cell growth (28). The exact
mechanisms that regulate these responses are still incompletely identified;
nonetheless these studies do highlight the importance of the ASM cell as an
interactive player in the remodeling process rather than being the passive
contractile partner as traditionally proposed.

Remodeling of the airway smooth muscle bundle: mechanisms

Although the mechanisms that regulate airway wall remodeling have thus far
been incompletely identified, there is likely a major role for airway inflammation.
Airway inflammation precedes airway remodeling in animal models of asthma
(29;30), and ASM is known to proliferate in response to numerous growth factors
and mediators that are released during allergic airway inflammation both \textit{in vitro}
and \textit{in vivo} (31). Nonetheless, both clinical and animal studies indicate that the
relationship between inflammation and remodeling is complex, and still
incompletely understood. The presence of airway inflammation in patients with
asthma is no guarantee at all for the occurrence of airway remodeling, and there
is no clear correlation between the degree of inflammation and the degree
of remodeling (18). Also, components of remodeling, including smooth muscle
thickening appear to be present already in young children (32-34) and there is
no clear relationship between age or duration of disease and the extent of ASM
thickening (19). Furthermore, although airway inflammation can be resolved
upon allergen avoidance in a murine model, remodeling persists, suggesting that
ongoing inflammation is not required to support the maintenance of the
remodeled airway wall (35). Collectively, although these studies point to an
important, probably indispensable, role for airway inflammation in initiating or
regulating the remodeling response, these studies also indicate that additional
mechanisms exist in the airway wall that are necessary to direct or maintain the
remodeling response. Moreover, these studies suggest that targeting
inflammation \textit{per se} may not be sufficient to reverse existing airway smooth
muscle remodeling, a contention supported by studies showing that
corticosteroid treatment prevents but does not reverse remodeling in allergen
challenged rats and mice (36;37).

In the next sections, we will discuss recent findings that underscore the
hypothesis that communication between different structural cells and
compartments of the airway wall is central to the development of remodeling and
may provide useful alternative drug targets for the treatment of smooth muscle
remodeling. These mechanisms include communication between the ASM, the airway epithelium, the airway parasympathetic nervous system and the ECM.

The airway epithelium

The airway epithelium forms the interface between the external environment and the airways (38). In asthma, the epithelial barrier is disrupted which contributes to AHR and inflammation associated with this disease via increased release of pro-inflammatory cytokines. In addition, (damaged) epithelial cells in asthma release a number of growth factors, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and VEGF, as well as acetylcholine (see below), which may contribute to airway remodeling in asthma by inducing ASM growth, ECM deposition and angiogenesis (38-41) (Figure 1). In this section, we will focus on the possible contribution of other epithelial processes, particularly alterations in L-arginine homeostasis, to airway remodeling in asthma.

Thus far, the role of L-arginine homeostasis in airway (patho)physiology has mostly been studied in the context of regulating airway (hyper)responsiveness. The epithelium is an important source of the bronchodilator nitric oxide (NO), which is produced by NO synthase (NOS) from the hydrolysis of L-arginine (42). Three NOS isozymes have been identified: neuronal (nNOS), endothelial (eNOS) and inducible NOS (iNOS). In the airway epithelium, nNOS and eNOS are constitutively expressed, whereas iNOS is particularly induced by proinflammatory cytokines during the late asthmatic reaction (43;44). The NO production is regulated by the substrate availability to NOS and alterations in the L-arginine homeostasis contribute to the pathophysiology of (acute) allergic asthma (45). Although levels of exhaled NO are elevated in asthmatics due the induction of iNOS (43;46;47), it has paradoxically been shown that a deficiency in bronchodilating (epithelium-derived) NO underlies the development of airway hyperresponsiveness in animal models of allergic asthma (48-54) and in asthmatic patients (55;56). This NO deficiency is caused by a decreased bioavailability of L-arginine to NOS isozymes (49;57;58), which also leads to uncoupling of the oxidase and reductase moieties within the iNOS enzyme (50). Uncoupled iNOS not only produces NO but also superoxide, leading to an efficient formation of peroxynitrite (59) (Figure 1), which induces airway hyperresponsiveness, epithelial damage, mucus hypersecretion and inflammation (60-63).
Figure 1 Putative role of altered L-arginine homeostasis in the airway epithelium in regulating remodeling of the ASM in asthma. The bioavailability of L-arginine to NOS isoforms is decreased in asthma, leading to a deficiency of bronchodilating NO as well as increased formation of procontractile peroxynitrite due to uncoupling of NOS. The NO deficiency may also contribute to the increased ASM mass in asthma, since NO is antiproliferative. Increased formation of peroxynitrite (ONOO-) could also contribute by causing epithelial damage. Reduced L-arginine bioavailability is caused by at least two mechanisms: i) inhibition of the cationic amino acid transporter by eosinophilic polycations, such as major basic protein (MBP), and ii) increased consumption of L-arginine by arginase, which is induced in asthma, presumably due to increased release of Th2-cytokines and growth factors. Increased arginase activity may directly contribute to the increased ASM mass via the production of polyamines and L-proline downstream from L-ornithine. See text for further detail.

A deficiency of NO could contribute to airway smooth muscle thickening. It has been shown that NO inhibits mitogen-induced proliferation of cultured human (64-66) and guinea pig (67) ASM cells. Scavenging of superoxide anions, thereby increasing the levels of authentic NO and inhibiting peroxynitrite formation, also decreased mitogen-induced human ASM cell proliferation (66). The downstream mechanisms of NO-mediated inhibition of cell proliferation have been studied in more detail in VSMC and involve cGMP-dependent repression of cell cycle promoting genes, including cyclin D1, and the induction of cell cycle inhibitors, such as p21Waf1/Cip1 (68). Also in VSMC, NO has been shown to inhibit the 26S proteosome, which regulates the degradation of cell cycle proteins, via S-nitrosylation (69), whereas cGMP inactivates p42/p44 MAPK and activates MAPK phosphatase 1 (68). Moreover, NO attenuates PDGF-induced activation of protein kinase B (PKB) and subsequent VSMC proliferation (70) and reduces embryonic fibroblast proliferation by inhibiting EGF receptor tyrosine kinase activity via S-nitrosylation (71,72). Taken together,
these findings suggest that a deficiency of (epithelium-derived) NO in asthma may also contribute to increased ASM mass in chronic asthma (Figure 1).

An important mechanism contributing to the L-arginine limitation to NOS and subsequent NO deficiency in asthma is increased consumption of the amino acid by arginase, yielding L-ornithine and urea (Figure 1). Two arginase isoforms have been identified, the cytosolic arginase I and the mitochondrial arginase II, and both are expressed in the airway epithelium (73). Arginase activity and/or expression of particularly arginase I are increased in a number of animal models of acute allergic asthma (for review see: (74)) and in human asthmatics (75-77). Increased arginase activity, which may involve Th2 cytokines (77;78), importantly contributes to the development of allergen-induced bronchial obstructive reactions (58), airway hypersensitivity in vivo and ex vivo (49;50;58;76;79) and airway inflammation (58). The functional significance of increased arginase activity in asthma is reinforced by reduced L-arginine levels due to inhibition of cellular L-arginine transport by eosinophil-derived polycations (50;80;81) (Figure 1). The release of NO by inhibitory nonadrenergic, noncholinergic (iNANC) neurons is also regulated by endogenous arginase (82). Moreover, the allergen-induced increase in arginase activity attenuates iNANC nerve-mediated NO release and ASM relaxation by limiting the L-arginine bioavailability (49). Thus, in addition to reduced ASM relaxation, alterations in L-arginine homeostasis in the iNANC nervous system may also contribute to airway remodeling via reduced synthesis of NO.

Increased arginase activity could also contribute to the pathophysiology of allergic asthma via increased synthesis of L-ornithine (Figure 1). L-Ornithine is a precursor of the polyamines (putrescine, spermidine and spermine) and L-proline, which are involved in cell proliferation and collagen synthesis, respectively (74;81;83-85). Polyamines induce the expression of genes involved in cell proliferation by promoting histone acetyltransferase activity and chromatin hyperacetylation (86); and polyamine synthesis is initiated by ornithine decarboxylase (ODC), which converts L-ornithine into putrescine (85). Both arginase and ODC are expressed in airway epithelial cells (87) and the expression and activation of both enzymes in the vasculature can be induced by growth factors, leading to increased polyamine levels (87-92). Growth factor-induced activation of ODC has also been observed in the airways (93). Interestingly, animal models demonstrate that arginase activity is increased in chronic asthma, underlying AHR in vivo (76) and ex vivo (94) by limiting the L-arginine availability to NOS. Moreover, elevated levels of polyamines have been detected in lungs of allergen-challenged mice (77) and in serum of asthmatic patients (95). These findings suggest that increased arginase activity may also contribute to the increased ASM mass in asthma via increased polyamine production. In support, transfection of VSMC with arginase I leads to elevated polyamine levels and increased cell proliferation (96). Since NO inhibits ODC via S-nitrosylation (97), allergen-induced deficiency of NO may contribute to the elevated polyamine levels in asthma.
In conclusion, altered L-arginine homeostasis due to increased arginase activity in the airway epithelium of asthmatics could contribute to airway remodeling via increased production of polyamines and L-proline downstream of L-ornithine as well as by limiting the substrate availability to NOS enzymes, leading to NO deficiency and enhanced peroxynitrite formation (Figure 1).

**The airway parasympathetic nervous system**

The airway parasympathetic system, in which acetylcholine is the primary neurotransmitter, has long been recognized for its role in bronchoconstriction and mucus secretion (98;99). However, recent studies in guinea pigs and mice have revealed that acetylcholine, by acting on muscarinic receptors, is involved in the regulation of ASM mass and phenotype, suggesting an important role of the airway cholinergic system in regulating responses associated with remodeling (100-102). Indeed, the regulation of neuronal release of acetylcholine appears to be highly facilitated by eosinophilic airway inflammation and acetylcholine appears to have postjunctional effects on ASM that could explain its action as a regulator of airway wall remodeling, notably thickening of the smooth muscle (103;104) (Figure 2A).

The release of acetylcholine from parasympathetic nerves is enhanced in allergic airway inflammation because several mechanisms exist that allow inflammatory mediators to activate the cholinergic system and because allergic airway inflammation facilitates the output of parasympathetic nerve endings (99;104) (Figure 2A). Afferent sensory nerve fibres, or C-fibres, play an important role in this regard, as they can be triggered by a variety of inflammatory mediators and by non-specific stimuli such as cold air. This results in the local release of tachykinins as well as the activation of a cholinergic reflex mechanism that facilitates the output of the vagal nerve, both centrally and locally in the airway parasympathetic ganglia (105;106). C-fibres are exposed due to epithelial shedding in asthma and have receptors for histamine, prostanoids, thromboxane A₂, bradykinin, serotonin and tachykinins (107;108). In conditions of damaged or stressed airway epithelium, the underlying mesenchyme can therefore be activated by this cholinergic reflex pathway, producing not only bronchoconstrictor responses but perhaps also responses associated with remodeling. The importance of this reflex mechanism is suggested by a recent study that showed that the majority of the bronchoconstrictor response to the inhaled thromboxane A₂ mimetic U46619 is prevented by vagotomy or by administration of the M₃ receptor selective ligand 4-DAMP (109). Furthermore, the airway hyperresponsiveness to inhaled histamine in ovalbumin sensitized guinea pigs after the early asthmatic reaction is markedly reduced by inhaled ipratropium bromide, indicating increased regulation of the cholinergic reflex in allergic airways disease (110). Such studies indicate that inflammatory mediators use the airway cholinergic system...
Structural components drive ASM remodeling to regulate a major part of their bronchoconstrictor response. In addition, several other mechanisms are at work in allergic airway disease that further induce the output of the vagal nerve. Inflammatory mediators including tachykinins, prostaglandins and thromboxane A2 facilitate neurotransmission in nerve endings and in the ganglia through effects on facilitatory prejunctional receptors (105;106). In addition, the prejunctional muscarinic M2 receptor which limits acetylcholine release under physiological conditions, is dysfunctional during allergic airway inflammation (98;111-113). Allergen-induced M2 dysfunction is regulated by eosinophils that are recruited to airway nerves, and secrete the allosteric muscarinic M2 receptor antagonist major basic protein (114). The above mentioned mechanisms collectively could well explain the increased role of the airway cholinergic system in airway hyperresponsiveness that is associated with loss of epithelial integrity. Since the airway cholinergic system also appears to regulate airway remodeling (100;101;115;116), the same may hold true for this pathological response.

Postjunctionally, there indeed are significant functional interactions between acetylcholine and growth factors that support ASM proliferation (Figure 2B). Muscarinic receptor stimulation by itself is not mitogenic to human ASM; however, in combination with growth factors such as PDGF or EGF, muscarinic M3 receptor stimulation augments the proliferative response to those factors, which likely involves the activation of multiple downstream effector pathways (117-119). In agreement with such an interaction, inhaled anticholinergics are effective in reducing ASM mass in guinea pigs that are allergen challenged, during which inflammatory mediators and growth factors are released, but have no effect on ASM mass in saline challenged controls (101). Muscarinic receptor stimulation cooperates with growth factor receptors to synergistically phosphorylate p70S6K and GSK-3, particularly in their late phase phosphorylation (2-4 h after stimulation) (118-121). P70S6K is required for ASM cell proliferation and hypertrophy and is activated upon phosphorylation, whereas GSK-3 is an anti-mitogenic and anti-hypertrophic kinase that is inhibited upon phosphorylation (Figure 2B) (118;122-125). Both actions of muscarinic receptors, which require the respective activation of PKC and p53 subunits as signaling intermediates (118;120;121), support therefore ASM growth. Effects of muscarinic receptors on smooth muscle phenotype marker protein expression (e.g. sm-MHC) could also be explained this way, as both p70S6K and GSK-3 regulate the expression of smooth muscle specific proteins (123;126). Direct activation of smooth muscle specific genes by muscarinic receptor stimulation has indeed been demonstrated (127;128), but the underlying signaling events and interactions with growth factors still need to be assessed in future studies. Interestingly, the study by Fairbank et al., (127) showed that amplification of MLCK expression by muscarinic receptor stimulation occurred only in the presence of mechanical strain, which highlights another potentially important mechanism for remodeling that is regulated by mechanical factors. Mechanical strain on ASM will result in ASM cell proliferation...
(127;129) and mechanical compression of the airway wall is sufficient to activate EGF receptors present in the airway epithelium (130;131). These studies raise the real possibility that bronchodilation, for example using anticholinergic agents, reduces airway remodeling at least in part via the reduction of mechanical stress and strain within the airway wall.

*Figure 2*  Interactions between the airway epithelium, the airway cholinergic system and the ASM regulates remodelling of the ASM bundle. A) Chronic airway inflammation facilitates acetylcholine release from the airway parasympathetic nerves, directly and via the activation of cholinergic reflex mechanisms, which are enhanced by the presence of damaged or stressed epithelium. B) As a result, increased acetylcholine release, in combination with growth factors and mediators released during inflammation coordinate cell responses in ASM associated with remodeling including smooth muscle specific gene expression and cell proliferation. The mechanisms responsible for these responses include phosphorylation of downstream signaling intermediates including p70S6K and GSK-3, resulting from muscarinic M3-receptor derived PI3K activation (via β3 subunits) and PKC activation respectively. See text for further detail.
In conclusion, the above mentioned studies have provided solid support for the hypothesis that airway inflammation can interact with neuronally derived acetylcholine to facilitate bronchoconstriction and ASM thickening. Also non-neuronal acetylcholine, released for example by airway epithelial cells or inflammatory cells may contribute to these processes, which needs to be established in future studies (104). Although this process is dependent on airway inflammation, the interactive role of the airway cholinergic system is considerable and appears to play a major role in regulating ASM mass and phenotype (Figure 2).

The extracellular matrix

The ECM is a dynamic structure that surrounds cells and provides the mechanical support required for airway structure and function. In the airway wall of asthmatics the amount and composition of the ECM is altered compared to healthy subjects. These changes are most eminent beneath the basement membrane and include increased deposition of collagens I, III and V, fibronectin, tenascin, hyaluronan, versican, biglycan, lumican and laminin α2/β2 as well as decreased expression of collagen IV, elastin and decorin (132-137) (Figure 3). Changes in the ECM have also been observed within and surrounding the ASM bundles. In patients with fatal asthma, the total amount of ECM within and surrounding the ASM bundles is increased, which was correlated with severity, but not duration, of asthma (20). This increase involves increased deposition of collagen I, fibronectin, hyaluronan, versican, biglycan, lumican and elastic fibres (138-140). A recent study, however, showed no changes in fractional area of collagen I or versican in the ASM layer (138). Interestingly, an inverse association between elastin expression and methacholine responsiveness has also been observed (10), suggesting that airway hyperresponsiveness is positively linked to the ECM expression in the ASM layer.

ASM cells are a rich source of ECM components, as shown by the production of collagens, fibronectin, laminins, perlecan, elastin, thrombospondin, versican and decorin by ASM (141-143), the expression of which is increased in response to profibrotic factors like TGF-β, CTGF and VEGF (144) (Figure 3). Interestingly, expression of CTGF in response to TGF-β by asthmatic ASM is increased compared to non-asthmatic ASM (22), identifying this factor as a potentially important contributor to ECM production in asthma. Altered ECM production by asthmatic ASM is also supported by findings showing an increased production of collagen I, perlecan and fibronectin (23;27), and a decreased production of laminin α1, chondroitin sulphate, collagen IV and hyaluronan compared to ASM derived from healthy subjects (27;145). In addition, increased expression of fibronectin, laminin γ1, perlecan and chondroitin sulphate by non-asthmatic ASM cells was observed after exposure to atopic serum, indicating that plasma leakage may contribute to increased ECM production by ASM in asthma (143).
ASM and ECM mutually affect each other to support abnormal ASM function as observed in asthma. A) In the asthmatic airways deposition of ECM proteins is increased, not only beneath the basement membrane but also within and surrounding the ASM bundle. Asthmatic ASM creates an altered ECM environment that facilitates increased contractile, proliferative and synthetic capabilities. B) Asthmatic ASM cells deposit increased amounts of fibronectin, which increases ASM synthetic function via a mechanism involving the α5β1 integrin. In addition, increased proliferation of asthmatic ASM has been shown to be dependent on the ECM, potentially also via a mechanism involving the α5β1 integrin. Laminins are critically involved in the expression of smooth muscle contractile proteins via the α7β1 integrin. Expression of this integrin has been shown to be increased by pro-remodeling factors like TGF-β.

Altered deposition of ECM proteins may alter mechanical properties of ASM, as well as the transfer of force between the ASM bundle and surrounding tissue (146). Next to their role in structural support, ECM proteins also regulate the function of the cells embedded therein. ECM proteins have been found to differentially regulate survival, migration, cytokine synthesis, maturation, contractility and proliferation of ASM cells (Chapter 3)(31;149). The alterations in the ECM profile produced by asthmatic ASM cells therefore have the potential to influence behavior and characteristics of the ASM cells (Figure 3). In support of this, studies on the effects of asthmatic ECM showed that culture of both healthy and asthmatic ASM cells on an asthmatic ECM enhanced proliferative responses (27). Similarly, increased eotaxin expression by asthmatic ASM is dependent on the ECM produced by these cells (23). This increased secretory response required interaction of the ASM with its ECM via α5β1 integrins (23), an integrin of which the expression can be increased in response to TGF-β in both nonasthmatic and asthmatic ASM (148) (Figure 3B). Preliminary findings from our laboratory also suggest an important role for integrins in ASM remodeling in vivo. Using a guinea pig model of chronic allergic asthma, we found that treatment with the integrin-blocking peptide Arg-Gly-Asp-Ser (RGDS),
Structural components drive ASM remodeling containing the RGD binding motif present in fibronectin, collagens and laminins (149;150), inhibits allergen-induced ASM hyperplasia, increased contractile protein expression and ASM hypercontractility, without effects on inflammatory responses (151). The normalization of allergen-induced hypercontractility also suggests a role for changes in ECM composition in regulating ASM contractility. Indeed, studies indicated that exogenously applied laminin-111 (laminin-1) maintains contractile ASM phenotype (Chapter 3)(152), whereas endogenously expressed laminin-211 (laminin-2) has been implicated in ASM maturation (153) and the induction of a hypercontractile ASM phenotype (Chapter 5). ASM maturation required activation of the laminin binding integrin α7 (154), of which expression is increased by TGF-β in both non-asthmatic and asthmatic ASM (155).

Collectively, these findings indicate that the ECM is not just an innocent bystander, but a component which can be actively regulated by the ASM, which in turn facilitates the abnormal ASM function as observed in asthma. These changes may be initiated by airway inflammation, but remain present in the absence of persistent inflammation. Intriguingly, these studies point to a dominant role of ASM – ECM interactions in the regulation of ASM remodeling and indicate that the muscle itself is capable of and in part responsible for creating an altered ECM environment that supports and maintains its increased contractile, proliferative and synthetic characteristics (Figure 3).

Conclusions

From numerous studies it is quite evident that ASM thickening is a prominent pathological feature in asthma, that contributes to an important extent to increased airway reactivity in patients. The mechanisms underlying this response remain elusive. Clearly, evidence is accumulating to indicate that the model, in which remodeling is due solely to the presence of inflammatory cells that secrete mediators and growth factors promoting cell proliferation and hypertrophy, is incomplete. Rather, the ASM layer is part of an active epithelial mesenchymal trophic unit that is activated during tissue injury and repair and driven by both changes in inflammatory cells and damaged epithelium. The damaged and stressed epithelium expresses increased levels of arginase, which reduces the presence of bronchodilatory and anti-proliferative NO, and promotes the presence of amino acids and polyamines that regulate smooth muscle remodeling. Furthermore, the damaged epithelium allows exposure of afferent sensory nerve endings that, together with the ongoing inflammation of the underlying airway wall, promotes the release of acetylcholine that acts as an important regulator of ASM remodeling via its actions on the postjunctional muscarinic M3 receptor. However, although epithelial cell changes and inflammation most likely play a major regulatory or initiating role, the studies summarized above also indicate that it is incorrect to assume that the underlying
mesenchyme, including the ASM, is a passive partner in the remodeling process. The ASM actively participates in the remodeling process by regulating inflammation through the secretion of chemokines and cytokines, by producing force on the airway wall during periods of inflammation that regulates gene expression and kinase phosphorylation via mechanisms of mechanotransduction, and by producing an ECM that supports its multifunctional role with respect to its proliferative, secretory and contractile capacities. Therefore, these studies call for a model of bi-directional rather than uni-directional communication between components of the airway wall, in which ASM thickening is controlled by several structural components, including the muscle itself.

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