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Targeting airway smooth muscle in airways diseases: an old concept with new twists

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Abstract

Airway smooth muscle (ASM) manifests a hyper-responsive phenotype in airway disorders such as asthma. ASM also modulates immune responses by secreting mediators and expressing cell-surface molecules that promote recruitment of inflammatory cells to the lungs. The aim of the current article is to highlight therapeutics that may modulate ASM responses in airway disorders and exacerbations.

Keywords

antibody therapy; asthma; bronchial thermoplasty; exacerbation; remodeling

Establishment of an inflammatory milieu in the airways, most likely contributing to the pathogenesis of asthma, occurs as a consequence of a myriad of cell types trafficking to the airway, directed by pathogens and mediators. In the context of such airway disorders, airway smooth muscle (ASM) primarily regulates bronchomotor tone and undergoes hypertrophy and hyperplasia; however, this tissue also contributes to the inflammatory milieu in airway disorders characterized by acute exacerbations. Although inflammatory mediators and pathogens activate receptors that are common among ASM, immune cells and epithelial cells, such mediators induce different cellular responses. Exposure of ASM to mediators released during airway inflammation or exacerbations of airway disorders amplifies the inflammatory capacity of the cell by increasing cell–cell interactions among ASM and immune cells in addition to modulating responses to contractile agonists and mediator release (Figure 1).

ASM growth & proliferation in asthma

Asthma is characterized by reversible airflow obstruction, but in some patients it can be irreversible owing to increases in ASM mass resulting from hypertrophy and/or hyperplasia. This increase in muscle mass could be related to severity and duration of disease. During a stable phase of asthma, the increased ASM mass likely contributes to decreased elasticity and increased obstruction of the airways, making normal air-flow levels difficult to achieve. During exacerbations of asthma, this increased mass may not only add to increased

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obstruction of the airways but also results in more sensitive airways, both of which worsen asthma symptoms. Proliferation of ASM is stimulated by growth factors and some, but not all, contractile agonists. Contractile agonists that stimulate ASM growth include histamine, endothelin-1, serotonin, α -thrombin, thromboxane A2 and leukotriene D4 [1,2]. Current therapeutics for asthma, including β_2 adrenergic receptor (β_2 AR) agonists and glucocorticoids, partially inhibit ASM growth. In human ASM, dexamethasone and fluticasone arrest cells in the G1 phase of cell division, thereby inhibiting ASM proliferation to some mitogens but not all. Other studies showed that inflammatory cytokines such as IL-4 and interferons (types I and II) also inhibit ASM proliferation by various downstream signaling mechanisms [3].

To potentially alleviate bronchoconstriction associated with increased ASM mass in airway diseases, such as asthma, bronchial thermoplasty has recently been reported as a therapeutic approach. The technique utilizes an instrument that is inserted in a bronchoscope that delivers a thermal injury that preferentially affects ASM. Surprisingly, the thermal injury has little long-term effect on other structural cells, such as epithelial cells, while decreasing methacholine responsiveness [4]. In a 5-year study performed in the UK [5], patients receiving bronchial thermoplasty exhibited no decrease in forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) over the 5-year period. Bronchial hyper-responsiveness to methacholine improved in those receiving thermoplasty in the second and third years of the study. Rates of exacerbations were also decreased in the thermoplasty group as compared with control patients treated with inhaled corticosteroids. In the randomized, controlled Research in Severe Asthma (RISA) trial, patients receiving bronchial thermoplasty exhibited decreased use of rescue medication, which manifested as a 15.8% increase in improvement in lung function (FEV_1), and reported a greater quality of life (asthma control scores) as compared with patients on traditional steroid and β -agonist therapy [6]. Trials of this technique are still underway to determine the safety and efficacy in treating patients with severe asthma that may be manifested by increased ASM mass. It is important to note that asthma is a disease not only of the larger airways, but also of the small airways. The long-term effects of bronchial thermoplasty may not ultimately have as significant an impact on decreasing airway hyper-responsiveness (AHR) or airway obstruction due to the limitations of its utility in airways within a given individual (Table 1).

Modulation of contractile responses has been the therapeutic target to directly affect AHR. A study of subjects with and without asthma that examined inspiratory volume noted that those without asthma dilate their airways to baseline measurements following methacholine provocation, whereas those with asthma were not only unable to dilate their airways well during deep inspiration, but the dilation following provocation was severely diminished. It was also shown that the airways of the subjects with asthma in the study recovered faster than the airways of the individuals without asthma. The contrasting results obtained between individuals with and without asthma may be attributable to characteristic differences in the ASM phenotypes. A study of isolated human ASM (HASM) noted increased velocity of force generation in asthmatic HASM as compared with nonasthmatic HASM (as reviewed in [7]). Antimuscarinic agents, including ipatropium, tiotropium and aclidinium bromide, serve to block receptors by binding mainly M_3 muscarinic receptors on ASM and abrogate the actions of acetylcholine or carbachol when applied topically. Ipratropium is a short-acting anticholinergic, whereas both aclidinium and tiotropium bromide are long-acting compounds for treatment of asthma and chronic obstructive pulmonary disease (COPD). A recent study by our laboratory showed that administration of aclidinium bromide decreased AHR as well as eosinophilia in a murine model of allergic airway disease [8]. In COPD patients, it was demonstrated that administration of tiotropium alone improved FEV_1 , but the combination of tiotropium with formoterol greatly improved FEV_1 , FVC, inspiratory capacity and reduced the necessity for use of albuterol rescue inhaler both during the day

and at night [9]. Studies also showed findings that patient symptom scores were improved during a 6-week treatment regimen in the group treated with both formoterol and tiotropium as compared with the tiotropium alone group [10]. It has also been demonstrated that M₃ receptor (M₃R) agonists synergize with growth factors to augment the proliferative capacity of the factors. Selective M₃R antagonists inhibit this synergism, and treatment of guinea pig ASM with tiotropium reduced allergen-induced increases in α -smooth muscle actin and myosin heavy chain (as reviewed in [11]).

Targeting of the β 2AR remains a mainstay in asthma therapy. Short-acting receptor agonists, such as albuterol for rapid relief of bronchoconstriction, or long-acting receptor agonists such as formoterol and salmeterol for extended control of airway hyper-reactivity, are widely used. While effective at bronchodilation, β 2AR agonists induce desensitization of the β 2 receptor that decreases the efficacy of such treatment. Modulation of the pathways downstream of the β 2AR has been an attractive approach for developing novel therapeutics. Targeting phosphodiesterases (PDEs), a class of enzymes that inhibit the breakdown of cAMP, can promote increases in cAMP levels. cAMP is an intermediary produced downstream from the β 2AR that promotes relaxation of ASM. Nonselective PDE inhibitors, such as theophylline have been used with some success, but directed targeting of PDE₄ isoforms has been examined as a more selective therapeutic. A recent study demonstrated that generalized inhibition of phosphodiesterases in asthmatic HASM had a 50% reduction in cAMP production following β 2AR-agonist stimulation. Expression and activity of PDE₄ were twice as high in asthmatic HASM as compared with that in nonasthmatic HASM [12]. Rolipram was a first-generation PDE₄ inhibitor that was found to decrease granulocyte–macrophage colony stimulating factor (GM-CSF) release of ASM exposed to either TNF- α or IL-1 β . While having an effect on the early phase of allergen-induced AHR, a more profound effect on the late-phase inflammatory response was identified with rolipram [13]. In a recent review of 23 randomized clinical trials, treatment with either cilomilast or roflumilast, both selective PDE₄ inhibitors, showed significant improvement in FEV₁ values over placebo control, as well as reduced the likelihood of exacerbation of COPD (Table 1) [14]. These therapeutics have off-target effects, and further study is necessary to evaluate the therapeutic benefits of these compounds in asthma.

ASM-mediated modulation of inflammation

Inflammatory disorders of the airways, such as asthma, and exacerbations are driven in part by secretion of mediators that not only modulate the responses of the immune system, but also significantly affect airway structural cells. TNF- α , a prominent cytokine present in the airways during allergic airway inflammation [15], evokes AHR [16]. In murine tracheal tissues, IFN- β and CD38 are required for TNF- α -induced AHR in response to carbachol [17]. Recently, evidence suggests that HASM derived from subjects with asthma expresses higher levels of TNF- α -induced CD38 expression in comparison to HASM derived from patients without asthma [18]. Interestingly, TNF- α synergizes with IL-1 β to promote β 2AR hyporesponsiveness, an effect ablated by selective cyclooxygenase-2 (COX-2) inhibition, providing evidence suggesting that the arachidonic acid derivatives serve to desensitize the smooth muscle to bronchodilation.

TNF- α also stimulates mediator production by ASM in addition to modulating airway responsiveness. IL-33 levels are elevated and co-expressed with TNF- α within tissue derived from endobronchial biopsies of subjects with severe asthma. TNF- α stimulation induces IL-33 in a time- and dose-dependent manner, and these levels synergistically increase with IFN- γ costimulation. Dexamethasone, however, fails to abolish TNF- α -stimulated IL-33 expression [19], thereby suggesting a role for this cytokine as a biomarker for glucocorticoid-insensitive, refractory asthma. Following stimulation with TNF- α and IL-1 β ,

ASM secretes eotaxin that activates RANTES, which is important in the recruitment of eosinophils in allergic airway inflammation, as well as IL-8, a known neutrophil chemoattractant. TNF- α stimulation increases secretion of IL-6, a cytokine that is thought to induce ASM hyperplasia, and modulates B- and T-cell proliferation and immunoglobulin secretion. Recently, we showed that coculture of ASM and epithelium enhances basal or TNF- α -stimulated IL-6 and IP-10 secretion [20].

Evidence suggests that ASM, both *in vivo* and *in vitro*, expresses thymic stromal lymphopoietin (TSLP), a cytokine thought to be necessary and sufficient to induce or maintain allergic airway inflammation. TNF- α stimulation of ASM induces mRNA and protein expression of TSLP, whose expression is dependent upon NF- κ B and AP-1 activity [21]. TSLP activates immune cells, such as dendritic cells, to elicit inflammatory mediator production and promote maturation of T cells into certain phenotypes. Interestingly, ASM also expresses functional subunits of the TSLP receptor that promote cytokine-induced release of IL-8, eotaxin and IL-6. Importantly, endobronchial biopsies from subjects with mild asthma express TSLP receptor on ASM [22]. Therapeutics directed towards this molecule/receptor have been utilized in animal models of allergic airway disease and have focused on modulation of dendritic and immune cell responses, but not effects on smooth muscle.

TNF- α stimulates airway eosinophilia and neutrophilia, maturation and differentiation of structural tissues, mucus hypersecretion, expression of cell adhesion molecules (CAMs) and T-cell activation. Studies performed in primates over two decades ago illustrated that use of anti-ICAM-1 antibodies inhibited allergen-induced AHR. TNF- α exposure of ASM promotes infiltration and adhesion of activated immune cells. TNF- α increases expression of CAMs on ASM, promoting increased interaction with/adhesion to inflammatory cells playing pivotal roles in the modulation of inflammatory responses associated with airway disorders [23,24]. ASM may also play a role in recruitment and potentiation of eosinophil survival through production of GM-CSF and IL-5, although the release of IL-5 by ASM remains controversial. ASM upregulation of VCAM-1 expression following TNF- α stimulation enhances neutrophil adherence [24]. Similarly, the potential for increased neutrophil recruitment to the airways following stimulation of ASM with TNF- α or IL-1 β may occur owing to IL-8 production.

Clinically, TNF- α has been proposed as a target for asthma therapy: in patients with severe asthma, elevated levels of TNF- α in bronchoalveolar lavage (BAL) fluid have been reported [25]. Antibodies targeting TNF- α , as well as a fusion protein of the type II TNF receptor with the Fc region of human IgG, are the therapeutics that have been used in clinical trials for treatment of asthma. In a study of subjects with asthma treated with infliximab, a monoclonal antibody directed towards TNF- α , subjects exhibited a decrease in mean diurnal variation in peak expiratory flow and decreased number of exacerbations, but little change in rescue albuterol use [26]. Etanercept, a TNF- α receptor/IgG fusion protein, significantly improved methacholine-induced AHR, increased FEV₁ and improved quality-of-life scores [27]. Another study utilizing etanercept as a therapeutic in the treatment of asthma noted that administration in refractory asthma produced a significant improvement in the methacholine provocation dose, as well as an increase in post-bronchodilator FEV₁ [28]. Morjaria *et al.* demonstrated in severe corticosteroid-resistant asthma that administration of etanercept failed to improve lung function, bronchial hyper-responsiveness or exacerbations when compared with the placebo-treated individuals [29]. Other than the effects of this therapeutic on the immune responses associated with asthma, anti-TNF- α therapy could act as a potential therapeutic to treat the contractile aspects of these diseases. Depletion of TNF- α may serve to decrease AHR by eliminating a source of cytokine-mediated sensitization of ASM to contractile agonists.

IL-1 β , along with TNF- α , modulates inflammatory pathways during exacerbations of asthma [15]. IL-1 β alone induces production of numerous mediators including prostaglandin-E₂, eotaxin, RANTES, GM-CSF, growth-related oncogene- α , epithelial neutrophil activating protein-78, monocyte chemoattractant protein-1, -2 and -3, IL-8 and IL-11 [30,31]. Additionally, many of the outcomes that IL-1 β elicits are additive to those stimulated by TNF- α . Some of the increased crosstalk between the cytokines can be attributed to the ability of IL-1 β to modulate TNF-RI and II expression [32]. IL-1 β acts cooperatively with TNF- α to increase ASM expression of the IL-17 receptor. IL-17 is a cytokine that enhances leukocyte influx and induces proinflammatory mediators in airway tissues [3]. IL-1 β alone, as well as in combination with TNF- α , increases production of matrix metalloproteinase (MMP)-12 and MMP-9 [33], MMPs known to degrade elastin, type IV collagen, fibronectin and laminin that promote cell migration, tissue repair and remodeling responses. Increases in extracellular matrix components may contribute not only to thickening of the walls of the airways, but also promote growth/proliferation of ASM. These components of the airways also can contribute to changes in mechanical properties of ASM, another mechanism to alter the contractile status of the airways. A study noted that exposure of HASM derived from subjects with asthma to IL-13, IL-1 β or TNF- α increases eotaxin 2.5–6-fold in comparison to HASM from healthy donors. They also showed that blocking of $\alpha 5\beta 1$ integrins abrogated the effect of IL-13-mediated eotaxin release from the asthmatic tissue (Table 1) [34].

IL-13 levels are increased in the airways of subjects with allergic asthma and IL-13 expression correlates with increased numbers of eosinophils in the airways and production of acid mammalian chitinase that modulates airway inflammation following allergen exposure. IL-13 produced by Th2 cells, mast cells, basophils and eosinophils induces hyper-responsiveness of rabbit tracheal smooth muscle and human precision cut lung slices to carbachol [35,36]. IL-13 and IL-4 induce MMP-1 production from ASM that can alter collagen type I matrix deposition and airway contractility [37]. IGF binding protein is degraded by active MMP-1, releasing IGF that stimulates ASM proliferation and migration. In response to IL-13, human ASM secretes eotaxin, which requires STAT6 activation [38]. IL-13 is also required for AHR, ASM hyperplasia and increases in IgE levels, as reported in murine transgenic models [39]. Thymus- and activation-regulated chemokine (TARC), a mediator promoting dendritic cell recruitment to sites of inflammation, evokes airway eosinophilia and AHR in murine models of allergen-induced AHR; TARC levels are also increased in the BAL from allergen-challenged subjects with asthma. IL-13, in combination with IL-4 and TNF- α , elicits TARC release from ASM, suggesting crosstalk among cytokine pathways in ASM. Using cultured ASM, IL-13, TNF- α and IL-1 β modulate G protein-coupled receptor-associated signaling pathways by enhancing agonist-induced calcium responses and/or altering cAMP production. Interestingly, IL-13 and TNF- α increase oxytocin receptor expression in human ASM, and oxytocin promotes force generation and airway narrowing. Since oxytocin is an acute-phase-response molecule, these data suggest that inflammatory cytokines produced during asthma can promote ASM receptor expression that then is activated by increased vascular permeability that delivers the ligand to the ASM [40]. Pitrakinra, an IL-4 variant that inhibits binding of both IL-4 and IL-13 to a high-affinity IL-4-binding receptor subunit (IL-4R α), attenuates signaling downstream of IL-4R. Administration of Pitrakinra prior to allergen challenge showed little effect on FEV₁ values, but significantly reduced the late-phase response to inhaled allergen and decreased the use of β -agonist rescue therapy [41]. This type of therapeutic may be useful for abrogating the sensitizing effect of IL-13 on ASM but would also inhibit the antiproliferative effect of IL-4. Such effects potentially negate each other if the increase in proliferation of ASM may be a greater contributing factor to the pathology of asthma as compared with the increase in ASM sensitivity imparted by IL-13. There have been few studies utilizing this therapeutic, and its potential for treatment of asthma remains unclear.

Cytokines and chemokines are produced by and directly modulate ASM function, but other inflammatory mediators, including lipid mediators, also influence the inflammatory environment created and maintained by ASM. Prostaglandins (PG), products of metabolism of arachidonic acid (AA) by COX enzymes, are mediators associated with different aspects of allergic airway inflammation. PGE₂ abrogates early- and late-phase responses to antigen challenge; in murine models of allergen-induced AHR, the lack of EP₃ receptors for PGE₂ increases airway inflammation and AHR to ovalbumin [42]. Other prostaglandins, such as PGD₂ and thromboxane, however, act as bronchoconstrictors that may enhance allergen-mediated airway eosinophilia [43]. Therapeutics antagonizing CRTH2, the receptor that binds PGD₂, have been in development and have shown promise in animal models of allergic airway disease.

Activation of other enzymes that generate metabolites of arachidonic acid is also impugned to modulate inflammatory airway disorders. Activation of 5-lipoxygenase (5-LO) enzyme generates leukotrienes that can be classified into two different classes, the cysteinyl leukotrienes (CysLT), which include LTC₂, LTD₄ and LTE₄, and the dihydroxy leukotriene, LTB₄. CysLT levels are increased in subjects with asthma during exacerbation or after antigen challenge [44]. In a murine model of virally induced AHR, montelukast, a CysLT1 receptor antagonist, blocks action of LTD₄ and reduces enhancement of AHR, eosinophilia and mucus hyperproduction upon reinfection with respiratory syncytial virus (RSV) [45], thereby illustrating a role for CysLTs, not only in allergic airway inflammation but also during viral exposures. Inhibition of 5-LO or antagonists of CysLT1 receptor improves FEV₁ measurements in asthma. A study comparing montelukast/fluticasone and salmeterol/fluticasone treatments showed that the rate of exacerbations of asthma was essentially identical when comparing the treatment regimens while montelukast/fluticasone treatment was effective in reducing peripheral eosinophil counts [46]. In a study of exercise-induced asthma, montelukast in combination with fluticasone significantly reduced the mean maximum percentage decrease in FEV₁ as well as time of response to albuterol rescue, as compared with salmeterol in combination with fluticasone [47]. The mitogenic effects of LTD₄ on ASM may also support the use of montelukast to attenuate ASM hyperplasia that may contribute to a reduction in airflow. Recently, our laboratory showed that early AHR observed during acute ozone exposure of murine airways is due to production of a cytochrome P450-dependent AA derivative, 20-HETE, which enhances airway responsiveness to carbachol-induced contraction. This was found to be steroid insensitive and independent of other lipid mediators derived from COX-dependent AA metabolism [36]. ASM can also produce lipid mediators. Studies showed that ozone induces IL-6 production via early enhancement of PGE₂ [20], and other factors, including bradykinin, IL-1 β , TNF- α , IFN- γ , cigarette smoke, lipopolysaccharide (LPS), mechanical stretch and cAMP-elevating agents potentiate PGE₂ production by ASM [48]. Collectively, these data suggest that production of PGE₂ can modulate events such as mast cell mediator release, epithelial barrier function, neutrophil and eosinophil chemotaxis, lymphocyte-derived IgE production and structural cell mitogenesis. Lipid mediators can have a profound effect on the contractile properties, as well as inflammatory capacity, of ASM in the context of airways disease.

IgE-mediated effects on ASM

IgE-mediated events in allergic airway disease have been mostly confined to the effects of the molecule on mast cell activation in initiation/perpetuation of the allergic response. HASM expresses transcripts as well as membrane-bound subunits for the high-affinity IgE receptor, Fc ϵ RI, and receptor α -chain immunoreactivity was found on smooth muscle from bronchial biopsies of subjects with asthma. Cross-linking of IgE elicited release of IL-4, IL-5, IL-13 and eotaxin and induced calcium mobilization that is necessary for smooth

muscle contraction [49]. By blocking IgE binding to its receptor, the effects of IgE crosslinking on mediator release from ASM are attenuated. Recently, Redhu *et al.* demonstrated that both TNF- α and IL-4 upregulate surface expression of the α -chain of the Fc ϵ RI on ASM, and pretreatment of ASM with TNF- α augmented the ability of IgE to induce eotaxin, IL-8, IP-10 and RANTES secretion [50]. Omalizumab, a biologic that inhibits the interaction of IgE with the Fc ϵ RI, has been used in treatment of allergic airway disease. This therapeutic is thought to inhibit allergen-dependent mast-cell activation by decreasing levels of circulating IgE, as well as downregulating Fc ϵ RI on circulating basophils, on skin mast cells and circulating dendritic cells. Studies showed that omalizumab decreased both early- and late-phase responses of patients to inhaled allergen but had little effect on bronchial hyper-responsiveness to methacholine [51]. Although administration of omalizumab had little effect on AHR, omalizumab is a useful therapeutic to inhibit the elaboration of cytokines induced by IgE cross-linking of Fc ϵ RI on ASM.

Cytokine-mediated steroid insensitivity in ASM

A mainstay therapeutic for asthma is use of glucocorticoids, including dexamethasone and budesonide. Apart from the inhibitory effects on ASM growth, steroids also induce secretion of extracellular matrix components from ASM both in the presence of profibrotic factors, such as TGF- β , as well as induce production of growth factors such as CTGF (as reviewed in [11]). In ASM derived from subjects with asthma, both budesonide and dexamethasone are unable to inhibit ASM proliferation as they do in normal ASM, which may be attributed to the type of matrix components these cells produce [52].

Severe asthma, as well as viral infections, either alone or in concert with underlying airway inflammation, exhibit insensitivity to steroid treatment that normally decreases airway inflammation. TNF- α that is found in BAL fluid from asthma, in combination with IFN- γ that is released during viral infection, induces steroid insensitivity. TNF- α synergizes with IFN- γ in the production of IP-10 through recruitment of CREB binding protein and subsequent increased RNA polymerase II activity [53]. Such synergism between these normally antagonistic pathways also increases RANTES, fractalkine and CD38 expression; however, IFN- γ itself inhibits IL-6, IL-8 and eotaxin production in ASM [3]. The increased cooperativity between these pathways may be due, in part, to an increase in TNF- α receptor expression by IFN- γ stimulation or vice versa [3]. Interactions between these pathways induce a steroid-insensitive state that is thought to be due, in part, to alternative splicing of a glucocorticoid receptor (GR) β -isoform. This GR isoform acts as a negative inhibitor of glucocorticoid action that is associated with steroid resistance in subjects with asthma [54]. Recent evidence in ASM suggest that steroid insensitivity may also be mediated through accumulation of interferon regulatory factor-1 (IRF-1), which occurs in a GR β -independent manner [55], and in cooperation with glucocorticoid receptor interacting protein-1 (GRIP-1). This protein acts as a coactivator for IRF-1, which binds GRIP-1 in excess to GR to induce a steroid-insensitive state [56]. Interestingly, there is a strong association between *IRF-1* polymorphism and childhood atopic asthma [57]. Studies also suggest that expression of IRF-1 increases following viral infection. This, in combination with the suppressive effect of IRF-1 on GR signaling, would potentially explain the reduced efficacy of steroids in viral-induced exacerbations of asthma [58]. Recent studies have suggested the use of vitamin D as a potential therapeutic for the treatment of steroid-insensitive asthma. Our laboratory demonstrated that treatment of ASM with vitamin D3 prior to costimulation of the cells with TNF- α and IFN- γ , mimicking the steroid-insensitive state seen in airways diseases, attenuated production of fractalkine and markedly inhibited RANTES release. Vitamin D also decreases levels of both RANTES and IP-10 following stimulation with TNF- α alone [59]. Studies examining the effect of vitamin D treatment of ASM demonstrated that a number of different genes were altered in expression, including those regulating growth,

proliferation, survival and genes potentially involved in airway remodeling [60,61]. IL-10, an anti-inflammatory cytokine produced by T-regulatory cells in response to steroids, is decreased in patients with steroid-insensitive asthma. Importantly, vitamin D3 restored T-cell IL-10 responses to steroids in subjects with asthma [62]. Further studies are required to determine the therapeutic potential of vitamin D for treatment of airway disorders such as asthma and COPD.

Effects of pathogens on ASM contribution to the inflammatory environment

Although viruses and bacteria induce inflammatory mediator release and evoke bronchospasm in healthy individuals, such pathogens markedly affect asthma and COPD morbidity and mortality. Pathogens evoke exacerbations of respiratory disorders through engagement of pattern recognition receptors such as Toll-like receptors (TLRs), thereby modulating cytokine release and contractile responses. ASM differentially expresses transcripts for most TLR isoforms [63]. As for fully functional receptors, ASM expression is restricted to TLR2, 3 and 4 with TLR3 being the most highly expressed [64]. Bacterial LPS, a TLR4 ligand, induces expression of VCAM-1 on ASM, facilitating adhesion of polymorphonuclear cells to ASM [65]. Peptidoglycan (a TLR2 ligand), dsRNA (a TLR3 ligand) and LPS induce release of IL-8 and eotaxin from ASM. TNF- α also increases expression of TLR2, an effect that is enhanced by costimulation with IFN- γ [64]. Furthermore, recent studies have shown that Der p2 activates the TLR2/MyD88 pathway in ASM cells to induce release of monocyte chemoattractant protein-1 and IL-6 [66].

As a viral exposure mimetic, Cooper *et al.* demonstrated in human precision cut lung slices that stimulation with poly(I:C) (a TLR3 agonist) induces production of a myriad of inflammatory mediators from airway tissue without inducing bronchoconstriction or enhancing carbachol-induced bronchoconstriction [35]. Stimulation of airway tissues with LPS, poly(I:C) or dual stimulation enhances contractility of tissue to bradykinin [67]. Additionally, stimulation of ASM with poly(I:C) recruits mast cells to ASM *in vivo* [68]. ASM stimulated with a combination of poly(I:C) and TNF- α or IL-1 β induces secretion of IL-6, IL-8, eotaxin and RANTES [30]. IP-10, IL-1 β , IL-6, IL-8 and IL-11 production by ASM is elicited by poly(I:C) or exposure to viruses including rhinovirus (RV) and RSV [30,69].

Respiratory viruses, including RV and RSV, have been identified as major causes of exacerbations of respiratory illnesses including asthma, COPD and cystic fibrosis (as reviewed in [70,71]). RSV infection has been linked in both the development and exacerbation of allergic airway disease; exposure to the virus induces airway hyper-reactivity and increased inflammation. In a rat model of RSV infection, RSV increased cholinergic-mediated ASM contraction. To address mechanisms by which RSV may predispose individuals to increased responses to allergic airway inflammation, a murine model of RSV infection prior to allergen challenge demonstrated that blocking of IL-13, which sensitizes ASM to contractile stimulation, reduced AHR and chemokine expression. Other investigators suggest that RSV-induced epithelial cell damage may expose nerves, thereby allowing substance P and neurokinin A to stimulate ASM contraction. In IL-8 receptor-deficient mice, AHR and TNF- α production following RV1B infection was reduced, suggesting a role for TNF- α and IL-8 in neutrophil-dependent AHR following viral exposure [72]. Development of therapeutics targeting RSV may reduce viral titers in both human and animal studies; however, a causal relationship in modulation of ASM responses remains unclear [73].

A definitive role for TLRs in eliciting production of inflammatory mediators or modulating ASM responses remains controversial, and it is likely that other mechanisms, including

intracellular viral recognition proteins such as protein kinase R, cell surface receptors, such as ICAM-1 (for major group RV) and the low-density lipoprotein receptor (for minor group RV), are involved. In a study of cohabitating individuals, one subject with asthma and one without, the person with the underlying asthma developed lower respiratory tract symptoms associated with development of a cold as compared with the counterpart without asthma [74]. A study in children demonstrated that persistence of RV infection in subjects with asthma induced persistent AHR and increased severity of exacerbations [75]. In human ASM, Grunstein *et al.* demonstrated that the effects of RV16, both active and UV-inactivated virus, stimulate IL-5 and IL-1 β secretion and that an ICAM-1-neutralizing antibody significantly decreased mediator levels following viral exposure [76]. Interestingly, viral-induced effects were not due to productive infection. In rabbit ASM, it was shown that RV16 exposure enhanced contractile responses to acetylcholine and impaired relaxation responses to isoproterenol, and that ICAM-1- or IL-1 β -neutralizing antibodies given prior to RV16 exposure abrogated the enhanced contractile responses. Oliver *et al.* demonstrated that RV16-induced IL-6 release from ASM derived from subjects with asthma was significantly greater than in normal ASM, a result that was not dependent upon viral replication in the cells [69]. These investigators also demonstrated that RV infection of nonasthmatic HASM significantly increased the deposition of fibronectin (40% increase; n = 12) and perlecan (80% increase; n = 14), while infection of asthmatic HASM cells significantly increased fibronectin (75% increase; n = 9) and collagen IV (15% increase; n = 9) [77]. Chronic RV exposure of ASM induces β 2AR hyporesponsiveness via secretion of IL-1 β . A study by Billington *et al.* also demonstrated that chronic exposure to either IL-1 β or RV16 significantly increases forskolin-induced cAMP production [78], suggesting that there may be a feedback mechanism in ASM cells to reduce the effects of virus towards a hyporesponsive state. Others showed that epithelial infection with RV16 modulates β 2AR activity in ASM through desensitization of cell-surface receptors. This effect could not be restored by treatment with either dexamethasone or fluticasone [79], which are steroids known to increase β 2AR expression on ASM [80]. Others have shown that RSV can productively infect ASM and significantly reduce isoproterenol-induced cAMP production [81], in a manner similar to RV modulation of β 2AR agonist-mediated responsiveness. Interestingly, Rho kinase inhibition suppressed AHR in a model of ovalbumin/RSV-induced AHR, suggesting that modulation of downstream signaling pathways by mediators produced during allergic airway disease/viral infection may be key to alleviating enhanced AHR due to viral exposure. Collectively, these studies also illustrate the potential of cytokines, whether released during inflammation or following viral exposure, to amplify responses to contractile agonists by modulating downstream pathways affecting calcium homeostasis in ASM.

In the past three decades, a number of compounds have been developed to treat respiratory infections induced by RV. Most of these compounds focus on binding to capsid proteins or inhibition of viral proteases to neutralize the activity of the virus. The compound R61837 was used in a clinical trial and reduced symptom scores and nasal secretions if administered prior to viral challenge [82]. WIN54954, another antiviral compound, was found ineffective, as assessed by antiviral activity and clinical outcomes, in an experimental RV exposure/infection trial [83]. Pirodavir was also used in studies with natural colds and was found ineffective at reducing symptom scores or duration of illness [84]. Pleconaril is a compound that prevents uncoating of picornoviruses and was shown to decrease duration of illness as well as symptom scores following experimental RV infection/exposure [85]. These types of drugs have yet to be used for virally induced exacerbations of asthma. Standard therapy of viral-induced exacerbations of asthma consists of corticosteroids and long-acting β 2AR agonists that reduce AHR in some, but not all, patients. The subset of patients with asthma whose disease is refractive to therapy drives a need for more targeted therapeutics for the management of exacerbations.

Summary

The responsiveness of ASM to an array of mediators, pathogens and injury is an important component of the inflammatory environment that is elicited in airway diseases or during exacerbations of these disease states. Not only does ASM respond to mediators released by other cell types, but in turn releases a host of mediators that regulate responses of the immune system. In addition to immune cells, ASM responses to pathogens, such as bacteria or viruses, may contribute to the pathophysiology associated with airway disorders. Molecular mechanisms by which ASM modulates these inflammatory responses may serve to be important and novel therapeutic targets for treatment of asthma and exacerbations of this disease. However, the effects of current therapeutics on outcomes, such as airway hyper-reactivity and lung capacity measurements, may not reflect a direct effect on ASM but may be a result of the interplay among a number of different cell types found in the airways.

Expert commentary

The expanse of studies that have been mentioned in this article allows us to make more than a causal link between ASM functions and worsening of airways diseases. Although the technique of abrogation of ASM *in vivo* utilizing bronchial thermoplasty is still in its infancy, the few studies that have been performed do show some promise as to its benefits, at least in the short term. There is a need to ensure long-term safety and efficacy of this technique, so the importance of follow-up studies further into the future with these patients is vital. The role of ASM as an immunomodulator, as well as its involvement in regulating bronchomotor tone, is also becoming clearer, as illustrated by the increasing number of studies demonstrating the inflammatory capacity of the cells. From this article, the utility of targeting ASM in inflammatory lung diseases to modulate sensitization of the tissue and contractile responses is potentially one of the most important therapeutic strategies to alleviate the early symptoms associated with disorders such as asthma.

Five-year view

Over the next 5 years, the hope would be to see designed and focused trials targeting molecular mechanisms underlying AHR related to ASM function. Such therapeutic trials would thereby support the hypothesis that targeting ASM is an important approach to treating one of the main causes of global respiratory disability for those suffering from asthma.

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Key issues

- Airway smooth muscle (ASM), the pivotal tissue regulating bronchomotor tone, serves an immunomodulatory role in airway disease.
- Bronchial thermoplasty improves exacerbation rates in severe asthma and may decrease ASM in asthma.
- Combination of β 2AR agonist with a corticosteroid is a mainstay therapeutic that differentially modulates ASM inflammatory responses.
- Modulation of contractile responses, either by blocking bronchoconstriction or stimulating bronchodilation, alleviates asthma symptoms.
- Pathogens and environmental pollutants evoke both contractile and immune responses from ASM.
- Vitamin D, a promising therapeutic for treatment of steroid-insensitive asthma, may abrogate viral exacerbations of the disease.

Figure 1. Pathogens, ozone and endogenous mediators induce exacerbations of asthma and chronic obstructive pulmonary disease through modulation of airway smooth muscle function
 Direct interaction of pathogens (virus/bacteria/allergen) or mediators produced by the body with ASM modulate inflammatory responses. Expression of pattern-recognition receptors by ASM allows these cells to respond directly to stimuli that are traditionally thought to affect immune cell function. Strong evidence suggests that ASM may modulate responses to these pathogens through release of mediators, interaction with/recruitment of immune cells, and amplified responsiveness of the cells to contractile stimuli.

ASM: Airway smooth muscle; CAM: Cell adhesion molecule; TLR: Toll-like receptor.
 Adapted with permission from [86].

Table 1

Novel therapeutics used in airways diseases.

Study (year)	Therapeutic approach	Outcomes	Ref.
Pavord <i>et al.</i> (2007)	Bronchial thermoplasty (32 subjects)	15.8% ↑ FEV ₁ ↑ asthma control questionnaire scores ↓ use of rescue medication	[6]
Cox <i>et al.</i> (2007)	Bronchial thermoplasty (56 subjects)	↓ number of exacerbations and rescue medication needed ↑ PEF and asthma control questionnaire scores No change in FEV ₁ or PC ₂₀ values	[87]
Castro <i>et al.</i> (2010)	Bronchial thermoplasty (297 subjects)	↑ asthma control questionnaire scores, PEF and rescue medication use 32% ↓ severe exacerbations 34% ↓ those experiencing steroid exacerbations No change in FEV ₁	[88]
Chong <i>et al.</i> (2011)	PDE ₄ inhibition (23 randomized clinical trials)	Significant improvement in FEV ₁ ↓ COPD exacerbation Small improvements in quality of life	[14]
Howarth <i>et al.</i> (2005)	Etanercept–TNF receptor fusion protein	↑ FEV ₁ , FVC and PEF values ↓ methacholine-induced AHR ↑ quality-of-life scores	[27]

↑ Increase in;

↓ Decrease in;

AHR: Airway hyper-responsiveness; COPD: Chronic obstructive pulmonary disease; FEV₁: Forced expiratory volume in 1 s; FVC: Forced vital capacity; PC₂₀: Dose of methacholine causing 20% fall in forced expiratory volume; PDE₄: Phosphodiesterase 4; PEF: Peak expiratory flow.