

Asthmatic airway hyperresponsiveness: the ants in the tree

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Airways from asthmatics have a propensity to narrow excessively in response to spasmogens (i.e., contractile agonists), a feature called airway hyperresponsiveness (AHR). AHR is an important contributor to asthma symptoms because the degree of responsiveness dictates the amount of airway narrowing that occurs in response to inflammation-derived spasmogens produced endogenously following exposure to environmental triggers, such as allergens, viruses, or pollutants. The smooth muscle encircling the airways is responsible for responsiveness because it constricts the airway lumen when commanded to contract by spasmogens. However, whether AHR seen in asthmatics is due to stronger muscle is equivocal. In this opinion article, I propose that environmental triggers and other inflammatory molecules released during asthma attacks contribute to AHR by increasing muscle force.

Airway smooth muscle force in asthmatic airway hyperresponsiveness

Asthma is a respiratory disorder caused by inhaled environmental triggers that affects 300 million people worldwide [1]. The symptoms of asthma result from excessive narrowing of the airways and are generally driven by inflammatory processes. Inflammation reduces airway luminal volume by causing airway wall edema, mucus hypersecretion, and, over a larger time scale, airway remodeling. Inflammation also occurs with the release of spasmogens (see [Glossary](#)), which are molecules that activate the airway smooth muscle (ASM). Upon activation, ASM generates force and strives to shorten. Owing to its circumferential arrangement around the airways, shortening of the ASM constricts the airway lumen ([Figure 1](#)). The effect of ASM on airway narrowing is fast-acting and can thus increase the resistance to airflow and the work required to breathe, not only seriously but also abruptly, as is typical during an asthma attack.

Within the lungs, the load impeding (opposing) ASM shortening increases progressively as the muscle shortens (the load is auxotonic). Thus, it seems logical that greater force generation by the muscle would lead to more shortening and concomitantly more airway narrowing. The molecular mechanisms responsible for generating force in ASM

are currently ill-defined. Although interactions between actin and myosin filaments, similar to those occurring in cardiac and skeletal muscle contraction, seem to be involved in ASM, mechanisms absent in other muscle types are now recognized to play important roles, such as cytoskeletal rearrangement and strengthening of the adhesion complexes ([Box 1](#)).

The amount of force generated by the ASM is obviously dependent on the amount of spasmogens that are simultaneously acting on it. Thus, more inflammation accompanied by a greater release of spasmogens (e.g., histamine or leukotrienes) would necessarily lead to more airway narrowing caused by ASM shortening. In addition, asthmatic individuals are known to be hyperresponsive to spasmogens, a feature called airway hyperresponsiveness (AHR). The degree of airway responsiveness is routinely assessed in certain laboratories by measuring the increase in airway resistance that occurs during a bronchoprovocative challenge with increasing doses of a spasmogen (usually methacholine). The two main features of AHR are hypersensitivity (i.e., a response to lower doses) and hyperreactivity (i.e., a greater response to a given dose). These observations have led some to hypothesize that AHR in asthmatics may be due to stronger ASM.

By contrast, a recent study suggests that both asthmatic and non-asthmatic ASM generate the same amount of stress (force/cross-sectional area) [2], and these data are in agreement with the conclusion of an earlier review on

Glossary

Bronchial ring: in airway smooth muscle jargon, a bronchial ring is a small segment of a bronchi, which has the shape of a ring.

Bronchoprovocation: a challenge with a substance that can trigger airway obstruction in some individuals; the challenge is usually by inhalation.

Deep inspiration: the inhalation of a volume of air that is greater than a normal tidal breath and that can go up to a maximal inhalation to total lung capacity.

Occupational agents: compounds, either an irritant or a sensitizer, to which a person is exposed during the course of performing their occupation.

Plasmalemma: the plasma membrane; a membrane composed of two layers of phospholipids and glycoproteins covering the surface of every cell.

Spasmogen: a mediator capable of causing muscle contraction. In the airways, a spasmogen is a mediator that induces a bronchospasm. It is also commonly named contractile agonist or bronchoconstrictor.

Tidal volume: the normal volume of air going in and then out of the lungs during breathing at rest.

Tone: a sustained contraction, usually triggered by a spasmogen, that can be either variable or constant in time.

Transpulmonary pressure: the driving 'force' that determines whether the air goes in or out of the lungs. It is the difference between the alveolar pressure and the pleural pressure.

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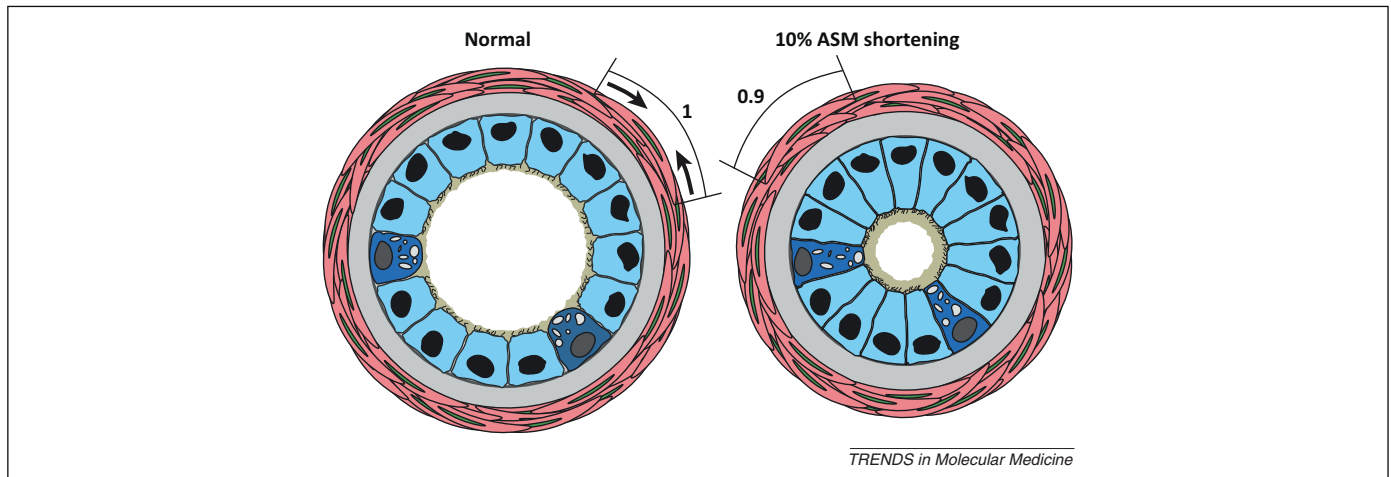


Figure 1. Airway smooth muscle (ASM) shortening induces airway narrowing. This cartoon is a representation of a cross-sectional area of an airway prior (left) and after (right) constriction. The ASM surrounds the airway. From the lumen (the inside, where air travels) to the muscle, the airway wall is composed of different layers, including the mucus (beige), the epithelium which is constituted predominantly of columnar epithelial cells (light blue) and goblet cells (dark blue), the basal lamina (thin darker gray) and the lamina propria (light gray). Once stimulated to contract in response to a spasmogen, the muscle strives to shorten. In this cartoon, narrowing of the lumen induced by reducing the length of every individual ASM cell by 10% can be visualized. The airway wall area was assumed to be incompressible, which gives a 'longer' shape to epithelial cells and thickens the airway wall layers of the constricted airway.

the matter [3]. A recent report actually suggested that the ASM of advanced asthmatics is hypocontractile [4]. Hence, the prevailing understanding is that the force generated by the ASM, at least when isolated and studied *ex vivo*, is not different between asthmatics and non-asthmatics. This may sound counterintuitive because asthmatics are generally hyperresponsive *in vivo*. An interim hypothesis can certainly be that the force may not be an important determinant of AHR in asthmatics. However, it is also possible that the conditions found *in vivo* are required to manifest the increased force.

Box 1. Strengthening of the adhesion complexes

New concepts defining the underlying molecular mechanisms driving ASM force development are currently emerging. The 'strengthening' of adhesion complexes, whose functions have been well described in migratory cells [58], seems to be required for optimal force generation in ASM. These points of junction between the contractile apparatus inside the cell and the extracellular matrix (ECM) outside the cell are excessively important for mechanotransduction efficiency; that is, the transfer of a molecular force generated inside the ASM cells by the successive pivoting of the myosin heads on the actin filaments to the tissue surrounding the ASM cells. The strength of the adhesion complexes is determined by the level of interconnectivity between the ECM, the plasma membrane, and the cytoskeleton of the cell. Once considered passive, recent studies suggest instead that the structure responsible for this interconnectivity can rearrange extensively upon ASM activation (reviewed in [59]). This level of plasticity may well be required to allow the ASM to function optimally over a wide range of lengths. For example, Zhang and Gunst [60] have shown that α -actinin translocates to the plasmalemma and binds the cytoplasmic tail of the $\beta 1$ integrin early after acetylcholine (ACh) activation, and blocking this interaction reduces tension generated in response to ACh. This and other recent findings [61] confirm that this interconnectivity between intracellular proteins, the plasma membrane proteins (e.g., integrins), and the ECM relies on dynamic processes that are activated by spasmogens. We shall henceforth consider this level of interconnectivity an integral part of mechanisms leading to force generation in ASM, and these new concepts certainly revolutionize the way we understand ASM contraction. Meanwhile, they open opportunities for the treatment of disorders that would benefit from blocking ASM contraction, such as asthma.

In vivo, the tracheobronchial tree of asthmatics is 'infested' by inflammatory molecules, which are absent *ex vivo* once the ASM is isolated and washed repeatedly before being studied. Thus, in my opinion, it is possible that the problem in asthmatics may not be an inherent (genetically determined) defect in the smooth muscle of the airway tree, but rather the omnipresence of inflammatory molecules that increase its contractility. In other words, 'it is not the tree but the ants in the tree' (Figure 2). I also suggest that it might be premature to discard ASM force as a factor contributing to the distinctive difference in airway responsiveness observed *in vivo* between asthmatics and non-asthmatics. Owing to citation restriction and the great deal of molecules discussed in this opinion article, it was impossible to cite all the influential papers published in this area [5].

Environmental triggers on ASM force

Several environmental triggers for asthma, such as allergens, pollutants, viruses, bacteria, and fungi, as well as a wide range of occupational agents have been shown to increase nonspecific (i.e., induced by an inhaled spasmogen) airway responsiveness. These inhaled environmental triggers are shown on the left side of Figure 2 within the airway lumen. Whether an increase in ASM contractility plays a role in the increased *in vivo* responsiveness induced by those environmental triggers is not always clear. The following section discusses the environmental triggers that have been shown to increase ASM contractility.

Pollutants such as the bacterial endotoxin lipopolysaccharide (LPS) [6], tobacco smoke [7], nicotine [8], ozone, nitrogen dioxide (NO₂), diesel exhaust, formaldehyde, acrolein, diisocyanates, coal dust, and welding fumes [9] have been shown to increase contractility of isolated airways or ASM. The house dust mite allergen *Dermatophagoides pteronyssinus* (*Der p 1*) also increases ASM force generation by a mechanism dependent on its cysteine protease activity [10]. *Ex vivo* contractility of bronchial rings derived from mice [11] or rats [12] sensitized and

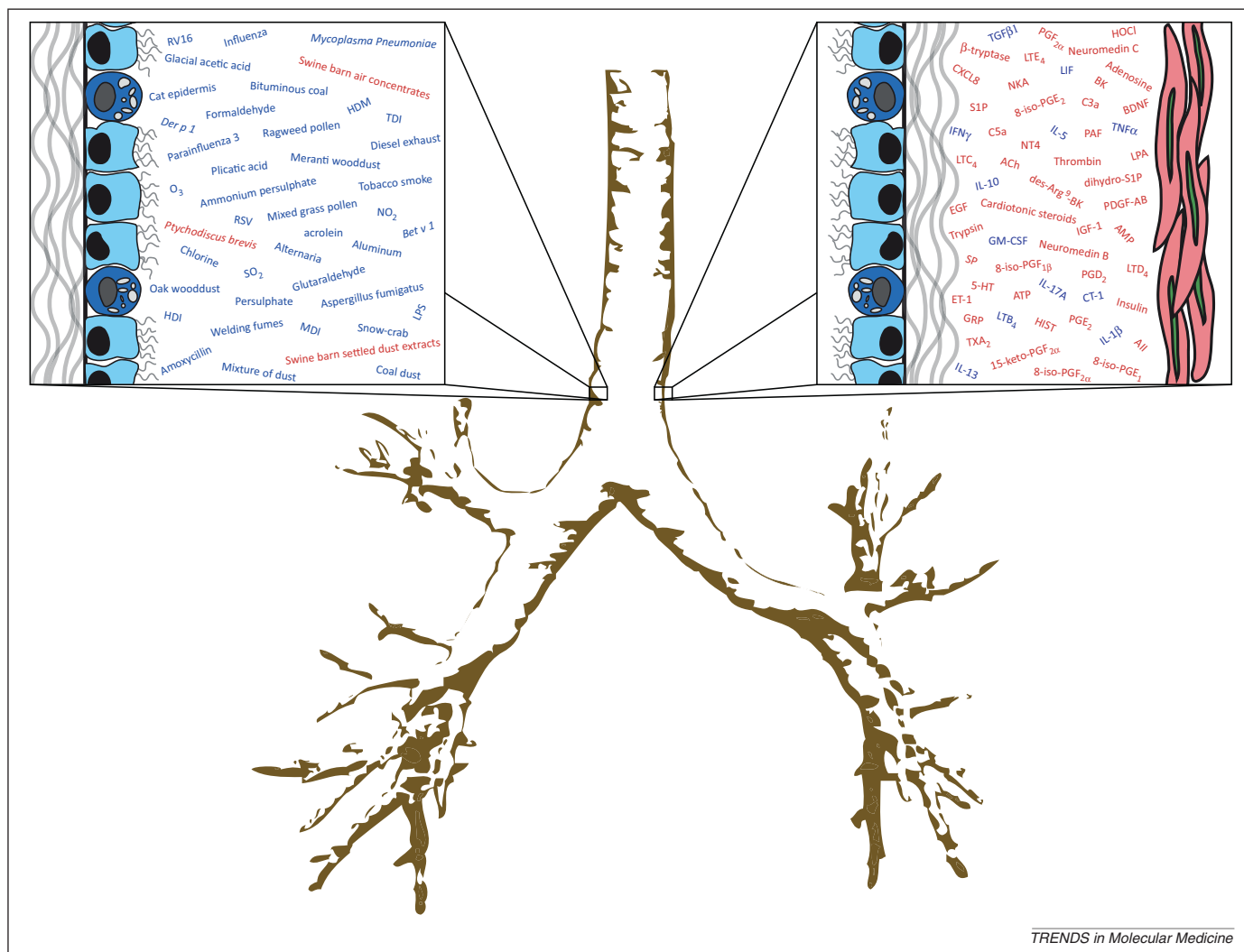


Figure 2. 'It is not the tree but the ants in the tree.' A myriad of inhaled irritants, allergens, and infectious agents, as well as many inflammatory molecules that are produced in response to those environmental triggers may contribute to airway hyperresponsiveness by increasing airway smooth muscle (ASM) force. All the molecules present on this figure have been shown to either induce ASM contraction (i.e., the spasmogens shown in red) or to potentiate the airway responsiveness to spasmogens (in blue) *in vivo* in either humans or animals, *ex vivo* in isolated ASM tissues, and/or *in vitro* using cultured ASM cells. For didactic purposes, the exogenously and endogenously derived molecules are shown in the airway lumen on the left and in the lamina propria (i.e., between the epithelium and the ASM) on the right. The shape of the tree was inspired by an anatomically correct human tracheobronchial tree [62]. Abbreviations: All, angiotensin II; ACh, acetylcholine; AMP, adenosine-5'-monophosphate; ATP, adenosine-5'-triphosphate; BDNF, brain-derived neurotrophic factor; Bet v 1, *Betula verrucosa* v 1; BK, bradykinin; CT-1, cardiotrophin-1; CXCL8, ligand 8 of the CXC subfamily of chemokines (also called IL-8); Der p 1, *Dermatophagoides pteronyssinus*; EGF, epidermal growth factor; ET-1, endothelin-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRP, gastrin-releasing peptide; HDI, hexamethylene diisocyanate; HDM, house dust mite; HIST, histamine; 5-HT, 5-hydroxytryptamine (also called serotonin); HOCl, hypochlorous acid; IGF-1, insulin-like growth factor-1; IFN γ , interferon γ ; IL, interleukin; LPA, lysophosphatidic acid; LPS, lipopolysaccharide; MDI, diphenylmethane diisocyanate; NKA, neurokinin A; NO $_2$, nitrogen dioxide; NT4, neurotrophin 4; LIF, leukemia inhibitory factor; LT, leukotriene; O $_3$, ozone; PAF, platelet-activating factor; PDGF-AB, platelet-derived growth factor-AB; PG, prostaglandin; RSV, respiratory syncytial virus; RV16, rhinovirus serotype 16; SP, substance P; S1P, sphingosine-1-phosphate; SO $_2$, sulfur dioxide; TDI, toluene diisocyanate; TGF β 1, transforming growth factor β 1; TNF α , tumor necrosis factor α ; TXA $_2$, thromboxane A $_2$.

challenged with ovalbumin is also enhanced, suggesting that other allergens, even without intrinsic proteolytic activity, may cause AHR by increasing the ability of the ASM to generate force. Finally, rhinovirus (specifically serotype 16, RV16), the virus primarily responsible for common colds and asthma exacerbation, and the virus mimetic polyinosinic:polycytidylic acid [13–15] were shown to potentiate ASM force development.

Together, these studies demonstrate that the force generated by the ASM in response to a given contractile stimulus is not stable but rather under the influence of a myriad of environmental irritants, allergens, and infectious agents. They also suggest that many environmental triggers may contribute to AHR by augmenting ASM contractility. In turn, the potentiating effect of environmental triggers on

ASM contractility is often reported to be mediated indirectly by provoking the release of endogenous molecules. For example, interleukin (IL)-13 [16], leukotrienes [17], and the sequential release of IL-5 and IL-1 β [13] were shown to be responsible for tobacco smoke-, ozone-, and RV16-induced hypercontractility, respectively. The effects of endogenous molecules that are either overexpressed in asthmatic lungs or released following exposure to environmental triggers on ASM contractility are discussed next.

Endogenously produced molecules on ASM force

Many spasmogens have been shown to be produced/released following exposure to environmental triggers. Histamine, leukotrienes, endothelin-1, the complement component C5a, bradykinin, prostaglandin D $_2$, and thromboxane A $_2$

Box 2. Force adaptation

Recent studies have demonstrated that a short period (~30 min) of tone induced by the presence of a spasmogen augments ASM strength over time [63]. Specifically, ASM was exposed continuously to the spasmogen ACh and ASM force generation in response to electrical field stimulations (EFSs) was monitored both before and after the induction of the ACh-induced tone (Figure 1). The force generated in response to EFSs before the addition of ACh was normalized to 1. The first EFS in the presence of tone was applied 1 min post-ACh administration and at 5-min intervals thereafter. On one hand, the addition of ACh immediately increased the total force (ACh-induced tone plus EFS-induced force), suggesting that ASM generates more force in response to two submaximal stimuli (ACh plus EFS) compared with EFS alone. This is simply an additive effect (bottom part of Figure 1). On the other hand, the increase in total force generated in response to the same two stimuli (ACh plus EFS) after the 1-min time point remains unexplained (upper part of Figure 1). This time-dependent increase in total force was coined ‘force adaptation’ [63]. ‘Force adaptation’ has recently been shown to occur in oscillating conditions that simulate breathing maneuvers (tidal breathing with or without periodic deep inspirations) [64]. This later reinforces the possibility that ‘force adaptation’ may occur *in vivo* when the ASM is not only exposed to spasmogens but also to the cyclical stresses that are acting on the airway wall and that are caused by the swings in transpulmonary pressure required for the movement of air in-and-out of the lungs.

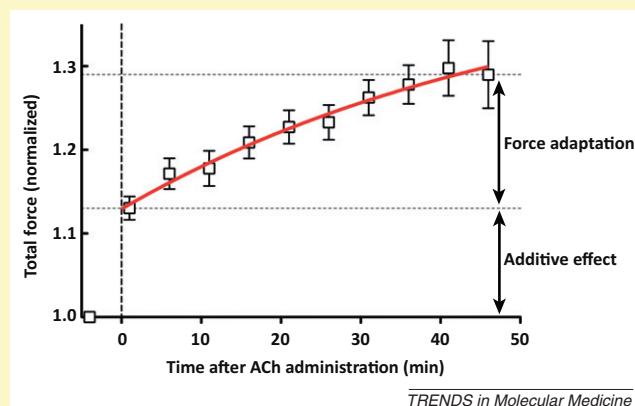


Figure 1. ‘Force adaptation’ is a time-dependent increase of airway smooth muscle strength that occurs in response to a continuous level of activation elicited by a spasmogen. The total force is the force generated by an electrical field stimulation (EFS) plus the exogenous acetylcholine (ACh); the later was used as the spasmogen. The total force prior the administration of ACh is the force generated by EFS alone. Mean and SEM are shown and a one phase association curve (red) was used to fit the data, $n = 17$.

are among the most studied. The spasmogens that have been shown to be overexpressed in asthmatic lungs are shown in red on the right side of Figure 2 within the lamina propria, the space between the epithelium and ASM. These spasmogens stimulate ASM contraction. Interestingly, much of the spasmogen synergize when used in combination so that the combined effect of two or more spasmogens leads to more force generation than the addition of their individual effects. For example, leukotriene C_4 [18], isoprostanes [19], lysophosphatidic acid [20], adenosine-5'-triphosphate [21], thromboxane A_2 [22], and sphingosine-1-phosphate [23] have all been shown to increase the contractile effect of other spasmogens. Molecular mechanisms underlying those synergisms were investigated in the studies above and seem to primarily involve processes that control ASM sensitivity to Ca^{2+} .

Spasmogens can also contribute to AHR by fostering a phenomenon called ‘force adaptation’ (Box 2). ‘Force adaptation’ is a gain in force acquired by the ASM during sustained exposure to a spasmogen, inducing a constant tone. The phenomenon is relevant to asthmatic AHR because airway tone is increased in asthma, based on the consistent finding that asthmatics demonstrate a greater response to drugs relaxing the ASM (e.g., bronchodilators such as β_2 agonists). Importantly, this gain in force caused by ‘force adaptation’ can be lost quickly upon abatement of tone by removing the spasmogen. Again, these observations strongly suggest that the force generated by the ASM is malleable and can change rapidly in response to various interventions.

There are also endogenous molecules that are not spasmogenic by themselves but increase the contractility of other spasmogens. A classic example is tumor necrosis factor (TNF) α , which has been shown to increase ASM contractility to the spasmogens histamine, leukotriene D_4 , bradykinin, des-Arg⁹-bradykinin, serotonin, neurokinin A, the thromboxane A_2 analog U46619, and the muscarinic agonists acetylcholine, methacholine, and carbachol. TNF α is actually used by a few groups as an *ex vivo* model of AHR [24,25]. IL-1 β is another classic example of a molecule shown to increase ASM contractility to several spasmogens, such as bradykinin, des-Arg⁹-bradykinin, substance P, serotonin, neurokinin A, and the muscarinic agonists acetylcholine and methacholine. Within the past decade, the list of molecules with this interesting property of enhancing ASM force generation has been growing considerably. The endogenous molecules produced in excess within asthmatic lungs that have been shown to potentiate the contractile effect of spasmogens are shown in blue on the right side of Figure 2. The last to be added to this list was IL-17A [26].

In some cases the molecular mechanisms by which these molecules potentiate the contractile effect of spasmogens are known, and they appear to differ from one molecule to another. Ca^{2+} sensitization via activation of the GTPase RhoA and the subsequent inactivation of the myosin light chain (MLC) phosphatase (MLCP) by Rho-associated, coiled coil-containing kinase (ROCK) has been shown to be involved in the potentiating effect of TNF α [27] on ASM contractility. Proficiency of this later signaling pathway to promote ASM hypercontractility is also enhanced by molecules capable of increasing the expression of RhoA, such as TNF α [28] and IL-13 [29]. Molecules such as interferon (IFN) β [30], IFN γ [31], TNF α [31], IL-1 β [31], and IL-13 [32] also increase ASM contractility by increasing the expression of the transmembrane protein CD38. Cytoplasmic Ca^{2+} mobilization that occurs following binding of spasmogens to their cognate G-protein-coupled receptor (GPCR) is enhanced by CD38 because it promotes the synthesis of cyclic adenosine-5'-diphosphate (ADP) ribose (cADPR), which, in turn, increases the probability of the ryanodine receptors (Ca^{2+} channels) on the sarcoplasmic reticulum of being open. There are other examples of how certain molecules overexpressed in asthma can augment ASM contractility. Increased contractility induced by upregulation of the contractile proteins α -smooth muscle actin, MLC kinase (MLCK), and smooth muscle myosin heavy

chain (smMHC) combined with actomyosin filamentogenesis and cellular hypertrophy by transforming growth factor (TGF) β 1 [33] and cardiotrophin-1 [34] are good examples. Upregulation of phosphodiesterase-4 activity induced by IL-13, which increases ASM contractility by decreasing the concentration of the relaxant cAMP, is another example [35]. Notably, these mechanisms are not mutually exclusive and the identification of common mechanisms is not out of sight, although more studies are clearly needed. Collectively, it suggests, once again, that the force generated by the ASM is not fixed but can change when bombarded by different external cues.

Implication for asthma treatments

Owing to asthma heterogeneity and the panoply of molecules that can be involved in the development of symptoms, it is not surprising that effective treatments target the two most common features of asthma: inflammation and ASM contractility. Glucocorticoids are especially efficacious because in addition to broadly buffering the inflammation, they reduce the expression of spasmogens and the other molecules that potentiate the contractile effect of spasmogens. It is hard to imagine that treatments for asthma would ever improve if we keep looking at asthmatics as a group of patients suffering from a single disease with a similar etiology. Therapies focused on a single molecule have generally been proven ineffective for treating asthma, even if they benefit some individuals. This is not surprising considering the great diversity of molecules involved, both within any given patient and between any two patients. If there is one thing we need to retain from the past few decades of research in this field, it is that there is no common molecular cause of asthma in humans. Therefore, the only way to further improve asthma treatments is to turn toward personalized medicine, which aims for individualized and targeted therapies. Obviously, this implies important additional cost for asthma diagnosis. Routinely categorizing asthmatics into well-characterized subgroups may be a promising avenue. Ample evidence now suggests that targeted therapies added-on to standard remedies can be effective in treating well-selected subgroups of asthmatics; this was the case for mepolizumab (an anti-IL-5 antibody) [36,37], lebrikizumab (an anti-IL-13 antibody) [38], tiotropium (a cholinergic antagonist) [39], and leukotriene receptor antagonists.

Complexity of airway responsiveness: ASM force and beyond

This article focuses exclusively on one contractile property by which ASM can contribute to airway narrowing *in vivo*, namely, force. Many other contractile properties of the ASM can affect the degree of airway responsiveness, such as contraction velocity and maximal ability to shorten, its stiffness and ability to tolerate and recover from oscillating strains that occur *in vivo* because of breathing, as well as its ability to relax upon spasmogen removal or in response to bronchodilators [40]. Alterations in any of those contractile properties potentially discriminate between hypo-, normo-, and hyper-responsiveness. Many exogenous and endogenous molecules have been shown to reduce responsiveness to bronchodilators – such as

RV16 [13], *Der p* 1 [10], LPS [6], TNF α [41], IL-1 β , IL-5 [42], IL-10 [43], IL-13 [44], TGF β 1 [45], sphingosine-1-phosphate [46], lysophosphatidylcholine [47], platelet-activating factor, platelet-derived growth factor-AB [48], and β -tryptase [49] – suggesting that those molecules can also compromise airway patency by decreasing the ability of the ASM to relax. Many molecules can also potentiate the contractile effect of spasmogens based on their ability to enhance ASM shortening (not necessarily by increasing ASM force). Selected examples include the effect of IL-13 on the contraction induced by leukotriene D₄ [50], the effect of TGF β 1 on ACh-induced contraction [51], and the effects of cardiotrophin-1 [34] and TGF β 1 [34,51] on the contraction induced by high concentrations of K⁺.

Although beyond the scope of this opinion article, airway responsiveness is also affected by many non-muscle factors [52]. Some of those factors simply alter the delivery and clearance of spasmogens to the ASM, such as vascular permeability and epithelial integrity [53,54]. Anything affecting the load impeding ASM shortening would also modify the level of airway narrowing for any given force generated. Edema, mucus hypersecretion, alterations of the surface tension, and many facets of airway remodeling are also known to impact airway responsiveness. With regard to remodeling, a recent study demonstrated that bronchoconstriction itself induced by the inhalation of methacholine can lead to thickening of the airway wall and hyperplasia of goblet cells [55]. This suggested that the level of airway responsiveness relies on a complex and interrelated interplay between muscle and non-muscle factors. Finally, airway narrowing heterogeneity that causes ventilation defects (patches of non- or hypo-ventilated area) due to airway closure, or near closure, can also cause airflow limitation and, consequently, contribute to AHR [56,57]. All in all, it is not just about ASM force.

Concluding remarks and future perspectives

AHR is a clinical feature of asthma and a good surrogate for the airway response that occurs in real life situations after spasmogens are produced endogenously following the inhalation of environmental triggers. Understanding AHR is thus a prerequisite to better understand asthma symptoms. Recent data confirming that asthmatic ASM is not inherently stronger than non-asthmatic ASM perplexed many investigators, and the message delivered in this opinion article is that sometimes it is not the tree but the ants in the tree. This is supported by mounting evidence suggesting that ASM force is not static (fixed) but rather malleable (adaptable). The recognized ability of both environmental triggers and endogenously produced molecules to increase ASM force may contribute to AHR seen in asthmatics.

It is important to note that most of the cited studies that investigated the effect of diverse mediators on ASM contractility were accomplished with animal tissues. Hence, these results need to be confirmed with human tissues. More research effort should also be directed toward identifying and describing in detail the molecular mechanisms governing the malleability of ASM force. The recent description of the phenomenon ‘force adaptation’ is a good demonstration that the strength of the ASM can change as

a function of time and in response to diverse interventions. Continued progress in our understanding of the molecular mechanisms driving increased muscle force, such as actin and myosin filamentogenesis, adhesion complex dynamism, Ca^{2+} sensitization, and alternative pathways potentiating Ca^{2+} signaling efficiency, could eventually lead to the discovery of molecules for which targeting can be salutary in the treatment of asthma.

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